GENETIC VARIATION IN *PRO-MELANIN-CONCENTRATING HORMONE* AFFECTS CARCASS TRAITS IN *BOS TAURUS* CATTLE

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By

Sarah Caroline Helgeson

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ABSTRACT

The purpose of this research was to determine whether genetic variation existed within *Bos taurus Pro-melanin-concentrating hormone* (*PMCH*), and whether this variation would affect carcass traits. *PMCH* had previously been shown to affect appetite and metabolism in rodent species, thus it was desirable to determine whether the gene had a similar effect in cattle, which could be interpreted based on carcass measurements of weight gain and fat production. Cattle *PMCH* was sequenced and an adenosine-to-thymine (*A>T*) single nucleotide polymorphism (SNP) was detected 134 bp upstream of the translational start site. The SNP alleles were determined to significantly affect carcass traits including average fat and grade fat in two populations of cattle, and shear force measurements in one population. The adenosine homozygotes were found to have the highest fat levels and the thymine homozygotes the least, while the heterozygous animals had intermediate fat levels. Shear force values in the one available population indicated that cuts of meat from the adenosine homozygotes were most tender, while cuts from the thymine homozygotes were least tender.

The SNP was also found to significantly affect tenderness and palatability of meat, as evaluated by a consumer taste panel. The meat from adenosine homozygotes was found to be most tender and palatable. These results could not be validated as this data was unavailable in additional populations.

The location of the SNP suggested that it may affect *PMCH* transcription rates. In silico examination of the different alleles indicated that the thymine allele introduces a novel transcriptional repressor binding site for Adenovirus E4 Promoter Binding protein

(E4BP4). Thus, it is believed that the SNP may affect transcriptional levels of the gene by reducing transcription rates in the presence of the thymine allele.

Cattle producers are expected to produce cattle with consistent amounts of lean meat and fat. Genetic testing of alleles found to affect fat production and meat tenderness traits are currently available to producers. A DNA test to select breeding stock based on *PMCH* alleles could be used in conjunction with other tests currently available to further improve carcass quality by selecting for animals with beneficial alleles at numerous genetic loci. Additionally, producers could make use of these findings to genetically sort cattle upon feedlot entry, maximizing the consistency of the finished beef product.

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LIST OF ABBREVIATIONS

α-MSH	Alpha-Melanin-Stimulating Hormone
˚C	Degree Celcius
aa	Amino acids
AGRP	Agouti-Related Peptide
Ala	Alanine
ANOVA	Analysis of variance
AP-1	Activator Protein-1
Arg	Arginine
bp	Base pair
BSE	Bovine Spongiform Encephalopathy
BTA5	*Bos taurus* chromosome 5
bZIP	Basic region leucine zipper protein
CBRH	Canadian Beef Reference Herd
cDNA	Complementary deoxyribonucleic acid
CRH	Corticotropin-Releasing Hormone
dH_2O	Deionized water
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
E4BP4	Adenovirus E4 Promoter Binding Protein
EDTA	Ethylenediamine tetraacetic acid
ER	Endoplasmic Reticulum
GAL	Galanin

GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GRE	Glucocorticoid Response Element
IRE	Interferon Response Element
LSM	Least Squared Means
Lys	Lysine
$MgCl_2$	Magnesium chloride
MC4R	Melanocortin-4 Receptor
MCH	Melanin-Concentrating Hormone
MCHR1	Melanin-Concentrating Hormone Receptor-1
MCHR2	Melanin-Concentrating Hormone Receptor-2
MGOP	MCH-Gene-Overprinted Polypeptide
μl	Microlitre
mM	Millimolar
MOET	Multiple Ovulation Embryo Transfer
mRNA	Messenger ribonucleic acid
NEI	Neuropeptide-Glutamic Acid-Isoleucine
NGE	Neuropeptide-Glycine- Glutamic Acid
NNPP	Neural Network Promoter Prediction
NPY	Neuropeptide-Y
NTS	Neurotensin
pmol	Picomoles
PCR	Polymerase Chain Reaction
PMCH	Pro-Melanin-Concentrating Hormone

poly(A)	Poly adenosine
POMC	Pro-Opiomelanocortin
PRNP	Prion Protein gene
QTL	Quantitative Trait Loci
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
SNP	Single Nucleotide Polymorphism
Thr	Threonine
TRE	Transcription Response Element
UTR	Untranslated region

1.0 GENERAL INTRODUCTION

In the beef cattle industry, animals that gain weight better than their counterparts are more desirable because they finish faster. Producers receive the most money when the animal has an optimal layer of fat, and are penalized for overly thin or overly fat animals. As a result of this, genes that influence the appetite pathway are of particular interest to the industry. Identification and characterization of the genes involved in appetite and metabolism aid producers in the selection of the best possible animals that will produce the most monetary gains.

Many neuropeptides have been shown to play a role in the regulation of appetite and metabolism in mammals, including Pro-Melanin-Concentrating Hormone (PMCH) (Shimada *et al.* 1998; Elliott *et al.* 2004; Gavrila *et al.* 2005). PMCH has been previously shown to participate in the regulation of food intake and metabolism in rodent species (Shimada *et al.* 1998).

PMCH was mapped to *Bos taurus* chromosome 5 (BTA5) (Stone *et al.* 2002). Further evidence that *PMCH* may play a role in fat production in cattle comes from the findings that quantitative trait loci (QTL) for backfat exist in the same region of BTA5 (Casas *et al.* 2000; Li *et al.* 2004).

These findings present *PMCH* as both a functional and positional candidate gene for the regulation of carcass traits in cattle. Within *PMCH* there may be allelic variation that may alter the rate of expression of the gene, or possibly the functional abilities of the

peptides produced, which may lead to phenotypic differences in carcass traits. If polymorphisms within *PMCH* are found to affect carcass traits, these findings can then be put to use by the cattle industry to aid in the selection of animals to produce of uniform, high quality beef.

This literature review will examine the structural properties of the *PMCH* gene and processing of the peptides produced, as well as discuss the known roles of the *PMCH*-derived peptides in the appetite pathway and their interactions with other appetite-regulating neuropeptides.

The objective of this thesis was to sequence *PMCH* and to characterize the gene in cattle, as well as to assess the conservation of cattle *PMCH* with that from humans, mice, and rats. A tissue expression profile was also to be determined, as this information was not yet available for cattle and may aid to further characterize the functions of PMCH. The identification of existing allelic polymorphisms within the gene was also to be undertaken, as well as determination of the possible functional effects these polymorphisms may have on cattle *PMCH*. Once polymorphisms were identified, differing alleles were analyzed with carcass trait data from the Canadian Beef Reference Herd (Schmutz *et al*. 2001) as well as a larger, unrelated validation population in order to determine whether these genetic polymorphisms affected carcass composition. The hypothesis was that genetic variations within *PMCH* were expected to influence fat deposition and carcass composition in beef cattle.

2.0 LITERATURE REVIEW

2.1 Gene products and localization

Pro-Melanin-Concentrating Hormone (*PMCH*) encodes three neuropeptides: Melanin-Concentrating-Hormone (MCH), Neuropeptide-Glutamic Acid-Isoleucine (NEI), and Neuropeptide-Glycine-Glutamic Acid (NGE) (Nahon *et al.* 1989). Additionally an alternately spliced product, MCH-Gene-Overprinted-Polypeptide (MGOP), has been shown to be produced in rats (Toumaniantz *et al.* 1996). *Pro-Melanin-Concentrating Hormone* consists of three exons and two introns (Figure 2.1; Breton *et al*. 1993b).

PMCH has been mapped in a number of mammalian species. In humans *(Homo sapiens*) *PMCH* is located on chromosome 12q23-q24 (Pedeutour *et al.* 1994), in mice (*Mus musculus*) on chromosome 10 (Kapfhamer & Burmeister 1994), in rats (*Rattus norvegicus*) on chromosome 7 (Nahon *et al*. 1992), and in cattle (*Bos taurus/indicus*) on chromosome 5 (Stone *et al.* 2002).

2.2 Peptide generation

Pro-Melanin-Concentrating Hormone (*PMCH*) is first transcribed as pre-pro-MCH, consisting of 165 aa (Figure 2.2). Cleavage of the signal sequence at $Glycine_{21}$

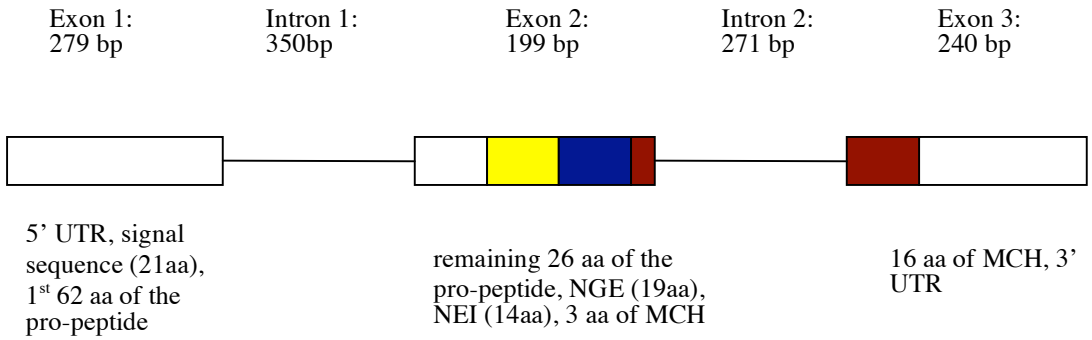

Figure 2.1 Depiction of human *PMCH* (Adapted from Breton *et al*. 1993a; Breton *et al.* 1993b; Nahon *et al.* 1993). Yellow shading indicates the coding region for NGE, blue indicates NEI, and red indicates MCH. The entire coding region consists of 165 aa.

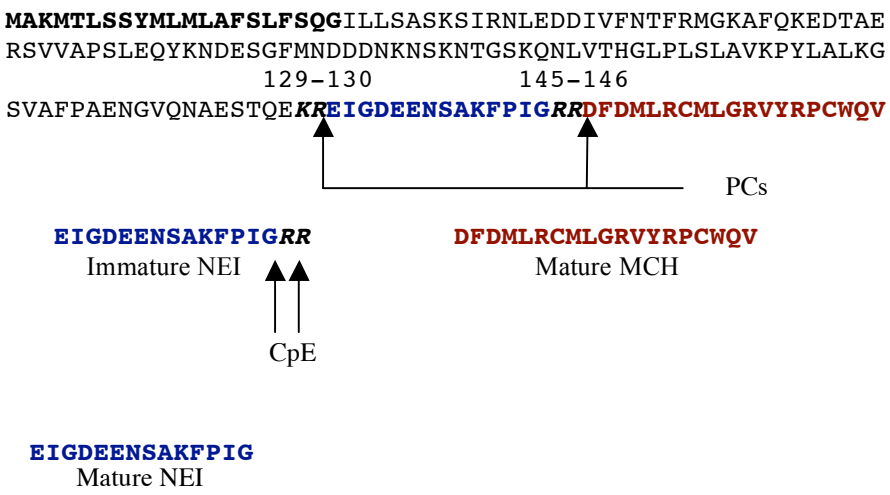

Figure 2.2 The proteolytic processing events required to produce mature MCH and NEI from PMCH (Adapted from Rovere *et al.* 1996 and Viale *et al.* 1999). The pre-pro-peptide consists of 165 amino acids. Cleavage of the signal sequence at $Glycine_{21}$ results in the formation of the pro-peptide, consisting of 144 amino acids. Subsequent cleavage at dibasic residues Lys_{129}-Arg_{130} and Arg_{145}-Arg_{146} releases mature NEI and MCH respectively. The 21 amino acid signal sequence is shown in bold, as are the dibasic amino acid cleavage sites. NEI sequence is shown in blue and MCH in red. PCs are Prohormone Convertases and CpE is Carboxypeptidase E.

results in the formation of pro-MCH (Nahon *et al*. 1989; Parkes & Vale 1992). Mature NEI is 14 aa in length and NGE is 19 aa. MCH is 17 aa in salmon and 19 aa in mammals. Based on the location of a putative cleavage site, MGOP is thought to consist of 14 aa (Toumaniantz *et al.* 1996).

Post-translational processing is required to liberate the mature peptide products from PMCH. The dibasic residues Lys_{129}-Arg_{130} separate NGE from NEI (Figure 2.2). An Arg_{145}-Arg_{146} site separates NEI and MCH. Cleavage at this site results in the production of mature MCH and immature NEI which still bears the Arg-Arg residues on the carboxy terminal. These residues are subsequently removed to release mature NEI.

MCH-Gene-Overprinted-Polypeptide (MGOP) was observed as an additional product in rats resulting from an alternate splicing event following transcription of *PMCH*. MGOP was found to consist of *PMCH* exons 1 and 3 and to utilize an alternate reading frame in exon 3 (Toumaniantz *et al*. 1996). This protein is thought to be the first described chimeric protein produced by altering the reading frame of an existing coding sequence in the mammalian neuroendocrine system. Translation of MCH in exon 3 utilizes the 3rd codon due to the presence of a single nucleotide at the end of exon 2, whereas translation of MGOP in exon 3 utilizes the 1st codon. Genomic coding sequences that utilize more than one reading frame to produce multiple peptide products from a single coding sequence are thought to be a source of diversity in some viral genomes, but are considered rare in mammalian genomes (Toumaniantz *et al*. 1996). Potential cleavage at a pair of Lysine residues (Lys_{110}-Lys_{111}) would result in the formation of a novel 14 aa protein. Structural similarities at the N-terminal between PMCH and MGOP reveal that both share the amino acid sequence that makes up the core

of the signal sequence, encoded in exon 1. These data suggest that MGOP may be directed toward the Endoplasmic Reticulum (ER) and processed through the regulated secretory pathway in the same way as MCH and NEI. Immunochemistry analysis in rats has revealed an MGOP expression pattern that is very similar to MCH, but at much lower levels (Toumaniantz *et al.* 1996). In support of this, another group discovered a transcript lacking exon 2 that was expressed in the thymus and testis of humans (Viale *et al*. 1997). The precise function of this alternate *PMCH* product remains unclear at this time, but may be generated as a result of the presence or absence of specific proteins involved in mRNA splicing.

2.3 Gene regulation

Various transcriptional regulatory mechanisms have been putatively identified in human *PMCH* sequence thus far. Activator Protein-1 (AP-1) binding sites have been putatively identified in the region upstream of the cap site and exon 2 in humans, as well as derived AP-1-binding sites in the promoter region and intron 2 (Viale *et al*. 1999). AP-1 binds to transcription response elements (TREs) to induce transcription. An interferon-γ response element (IRE) and a partial glucocorticoid response element (GRE) have also been putatively identified in the 5' region of rat and human *PMCH* (Viale *et al*. 1999). IRE sites are responsible for transcription regulation in macrophages. A GRE site would allow for *PMCH* transcription induction in the presence of glucocorticoids. This evidence suggests that the *PMCH* promoter may be responsive to a number of physiological signals.

An additional regulatory mechanism involves complementary sequences that may form hairpin loops in the mRNA sequence. A hairpin structure at the 5' end just upstream of the start codon is believed to be important in regulating translation initiation by directing ribosome pausing, or abolishing translation altogether (Breton *et al.* 1993a). Another hairpin at the 3' end spans the MCH stop codon and may influence the stability of the mRNA. Changes in the length of the *PMCH* mRNA transcript have been observed in mice, owing to variations in the length of the poly(A) tail (Breton *et al.* 1993a). This data was further supported by findings in rats showing variations in poly(A) tail length (Hervieu & Nahon 1995). The shortest poly(A) tails were observed in *PMCH* species localized in the peripheral organs, while the longest poly(A) tails were observed in the brain. The poly(A) tail has been associated with 3'end processing, splicing, and nucleus-cytoplasm transportation from the nucleus, as well as translation efficiency, mRNA stability, and subcellular location in the cytoplasm (Breton *et al.* 1993a). Cumulatively, these findings suggest that regulation of mammalian *PMCH* may occur at both the transcriptional and translational level.

2.4 Tissue expression profiles of *PMCH* in other species

PMCH mRNA has been detected in the human hypothalamus as well as the spleen, thymus, brown fat, duodenum, adrenal gland, bone marrow, testis, and lymphatic gland, but was absent in aorta, kidney, white fat, bladder, stomach, and pylorus (Viale *et al*. 1997). *PMCH* mRNA has also been identified in human immune cells (Verlaet *et al*. 2002) and skin (Hoogduijn *et al*. 2002). In rats, *PMCH* mRNA has been detected in the hypothalamus, stomach, intestine, and testis (Hervieu & Nahon 1995), as well as in the

adipocytes (Bradley *et al*. 2000). In mice *PMCH* mRNA has been found in the heart, intestine, spleen, and testis of both adults and neonates (Breton *et al*. 1993a). The possible function of *PMCH* in tissues other than the brain is not known.

2.5 Receptors

MCH has exclusive receptors found throughout the body. MCH-Receptor-1 (MCHR1) was found in all mammals studied whereas MCH-Receptor-2 (MCHR2) has only been found functional in dog, ferret, rhesus, and human (Tan *et al*. 2002). There are truncated versions of MCHR2 present in the rabbit and guinea pig, while MCHR2 is completely absent in rodents such as rats and mice. It is thought that an initial duplication of the MCH receptor gene occurred before the divergence of vertebrates, and that rodents may have lost *MCHR2* following the divergence of rodentia (Logan *et al.* 2003). The precise role of *MCHR2* is not known at present due to the lack of an appropriate animal model.

A functional role for MCHR1 in appetite regulation has been suggested. Mice in which *MCHR1* has been disrupted are lean and hyperactive, and also exhibit hyperphagia and increased metabolism (Marsh *et al*. 2002). Antagonism of MCHR1 has been shown to prevent MCH-induced food intake (Morens *et al*. 2005). Fasting or genetic leptin deficiency has been shown to upregulate *MCHR1* expression (Kokkotou *et al*. 2001). Mutations in *MCHR1* have been detected in association with obesity in a cohort of German children (Wermter *et al*. 2005). Analysis of Single Nucleotide Polymorphisms (SNPs) found in *MCHR1* have led to the suggestion that this receptor may play a role in

the polygenic form of severe early onset human obesity (Bell *et al.* 2005). Thus, MCHR1 appears to be an important mediator of the effects of MCH on appetite and body weight regulation in rodents.

2.6 Peptide functions

2.6.1 Pigmentation

MCH is the most extensively studied protein derived from *PMCH*. MCH was first discovered in teleost fish and was named as a result of the finding that, in lower vertebrates, MCH functions as an antagonist to Alpha-Melanin-Stimulating Hormone (α-MSH) to control scale pigmentation (Kawauchi *et al.* 1983). MCH concentrates the melanophores in fish scales to give the overall appearance of lighter pigmentation while α-MSH disperses the melanophores, resulting in the appearance of darker pigmentation.

The role of MCH in mammalian skin pigmentation has not yet been confirmed, although evidence has been presented that suggests a potential role. Mice lacking MCH or MCHR1 appear phenotypically normal (Shimada *et al.* 1998; Marsh *et al.* 2002), however autoantibodies against MCHR1 have been found in human patients with the depigmentation disorder Vitiligo (Kemp *et al.* 2002). Vitiligo occurs as a result of the destruction of functional melanocytes in the skin. This evidence suggests that MCH and its receptor may play a role in skin pigmentation of mammals.

2.6.2 Appetite

Evidence supporting the role of MCH in the appetite pathway was obtained from knockout mice that were phenotypically lean, exhibiting decreased food intake and

increased metabolism (Shimada *et al.* 1998). In an additional study, it was shown that MCH knockout mice appeared normal initially, but developed reduced body weight and decreased leptin expression after 7 weeks of age (Alon & Friedman 2006). These mice were found to have decreased fat mass, decreased body length, and decreased lean mass, characterized by hypophagia and increased energy expenditure. On the basis of this evidence, it appears as though MCH is capable of causing changes in energy metabolism when disrupted in mice.

MCH administration in mice leads to obesity and insulin resistance due to increased food intake and reduced metabolism (Figure 2.3; Ito *et al.* 2003). These MCH-induced increases in food intake have been shown to be a result of increased meal number, size, and duration, and were shown to be preventable by prior administration of a MCH receptor antagonist (Morens *et al.* 2005). The effects of MCH administration may not necessarily be long-term however. It was shown that MCH injection directly into the brain induced appetite in satiated rats, although tolerance developed after six days of treatment (Rossi *et al.* 1997). There were no overall changes in body weight observed and the conclusion was drawn that MCH was not involved in long-term regulation of body weight maintenance.

In a study involving mice homozygous for an overexpressed MCH gene, it was shown that two-fold overexpression of MCH resulted in a 10% increase in food consumption and a 12% increase in body mass by thirteen weeks of age compared to normal littermates (Ludwig *et al.* 2001). Additionally, an alternate strain of laboratory mice that were heterozygous for the overexpressed gene also became obese on a standard diet. These results tend to support long-term effects of MCH overexpression and

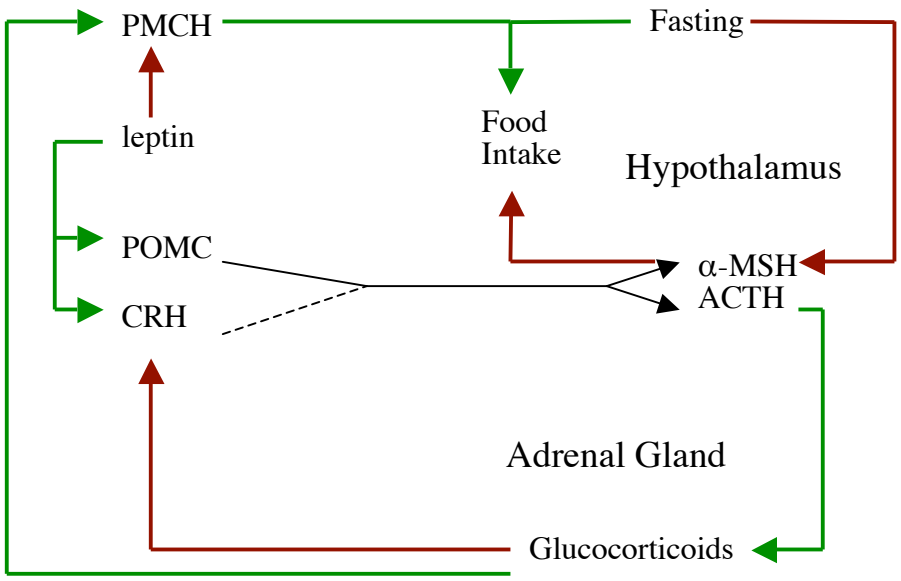

Figure 2.3 The influence of leptin, stress, and fasting on several appetite-regulating genes (Based on the findings of Ito *et al.* 2003; Qu *et al.* 1996; Ludwig *et al.* 1998; Drazen *et al.* 2004; Sahu 2004). Leptin increases the expression of appetite-reducing neuropeptides and decreases the expression of appetite-decreasing neuropeptides in the hypothalamus. Fasting increases the expression of MCH, whereas stress decreases the expression of MCH and increases expression of *POMC* which is processed by CRH to produce α-MSH and ACTH. During stress, ACTH acts on the adrenal gland to cause the release of glucocorticoids which then down-regulate expression of *CRH*, and increase expression of *PMCH* in a regulatory feedback loop. Green arrows indicate up-regulation, while red arrows indicate down-regulation.

emphasize the importance of gene interactions in a different genetic background. These findings also contrast those found in animal models where MCH was administered, suggesting that administration of exogenous MCH has shorter-term and less potent effects than increasing endogenous expression. This evidence suggests that overexpression of MCH may lead to the onset of obesity.

2.7 Physiological regulation

Physiological signals are key regulators of neuropeptide expression. Starvation and obesity have been shown to impact neuropeptide regulation (Figure 2.3). Fasting has been demonstrated to increase expression of MCH in mice, rats, humans, and sheep (Qu *et al*. 1996; Herve & Fellmann 1997; Gavrila *et al*. 2005; Henry *et al*. 2000). In humans, serum MCH levels have been shown to increase in correlation with fat mass and response to fasting, but were not associated with total caloric intake (Gavrila *et al*. 2005). These effects could be annulled by prior administration of leptin. Conversely, overeating has been shown to decrease MCH mRNA expression (Presse *et al*. 1992). This evidence suggests that MCH expression is increased during periods of nutritional deprivation.

Stress and glucocorticoids appear to influence MCH expression in the hypothalamus (Figure 2.3). During states of stress, ACTH acts on the adrenal gland to cause the release of corticosterone, a prominent glucocorticoid. Glucocorticoids cause the breakdown of skeletal muscle and adipose tissue to release substrates for gluconeogenesis in the liver. Stress has been shown to decrease the expression of *MCH* in mice and rats (Parkes & Vale 1992), as well as to have the opposite effect on CRH and ACTH release (Presse *et al*. 1992). Interestingly, MCH administration has been shown

to block the stress-induced rise of plasma corticosterone which is thought to be mediated by the inhibition of ACTH (Ludwig *et al.* 1998), indicating that MCH may have an inhibitory effect on ACTH. Adrenalectomy, the removal of the adrenal gland, has been shown to decrease *PMCH* expression in the hypothalamus, whereas the administration of exogenous glucocorticoids were shown to restore *PMCH* expression to baseline levels (Drazen *et al.* 2004). These findings indicate that glucocorticoids stimulate *PMCH* expression. Conversely, glucocorticoids have been shown to have inhibitory effects on both CRH and POMC (Jingami *et al.* 1985). One proposed mechanism for the actions of MCH during states of stress is that the stress-mediated decrease in MCH is counter-acted by the release of glucocorticoids acting as a positive regulator of expression (Nahon *et al.* 1993).

Cold exposure has been shown to increase hypothalamic MCH expression, leading to the hypothesis that MCH may participate in the process that allows for efficient use of energy for heat production during adaptation to cold weather (Pereira-da-Silva *et al.* 2003). Heat production must be efficient if vast amounts of energy are not to be wasted during acclimatization.

2.8 Interactions of MCH with other peptides involved in appetite regulation

The hypothalamus is thought to be the primary site of integration for the physiological factors responsible for maintaining energy homeostasis (Sahu 2004). Leptin is a central regulator of feeding behavior as a result of the ability to regulate the expression of several other peptides (Figure 2.3). Leptin deficiency has been associated with severe obesity in both rodents and humans (Sahu 2004). Leptin increases the

expression of anorectic peptides such as *Neurotensin* (*NTS*), *Pro-opiomelanocortin* (*POMC*), and *Corticotropin-Releasing Hormone* (*CRH*), while decreasing the expression of orexigenic peptides such as *Neuropeptide Y* (*NPY*), *Agouti-related Protein* (*AGRP*), *Galanin* (*GAL*), and *MCH* (Figure 2.3; Sahu 2004).

MCH was first implicated as a regulator of food intake in mammals when it was discovered that *MCH* mRNA expression was increased 2-3-fold in *leptin* deficient *ob*/*ob* mice when compared with normal mice in both fed and fasted states (Qu *et al*. 1996). Furthermore, disruption of *MCH* in *ob*/*ob* mice resulted in a lean phenotype as a result of increased resting energy expenditure and locomotive activity, as well as improved thermoregulation (Segal-Lieberman *et al*. 2003). MCH expression has been shown to stimulate *leptin* mRNA expression and secretion (Bradley *et al*. 2000) in a regulatory feedback loop. Leptin was shown to fully antagonize MCH-induced food intake, indicating that MCH acts via a leptin-sensitive pathway (Sahu 1998). The exact mechanism of this antagonism remains to be elucidated.

There is clearly an interactive relationship between leptin and MCH that functions to mediate food intake and nutritional status. Leptin's role in the regulation of *POMC* is particularly critical to regulate feeding behavior. Leptin increases *POMC* neuronal activity, which is then processed to release α-MSH (Sahu 2004). It has been shown that MCH knock-out mice have decreased *POMC* expression (Shimada *et al*. 1998). MCH was shown to have opposing actions relative to those of α-MSH. MCH was shown to increase food intake in rats and to decrease plasma glucocorticoid levels while α-MSH was shown to have the opposite effects (Ludwig *et al*. 1998). The mechanism for antagonism of α–MSH by MCH is not known at this time but may be as a

result of control over *POMC* expression. Decreased *POMC* expression would lead to decreased α-MSH production. This suggests that without a signal to induce food intake, the signal to reduce food intake may be suppressed.

2.9 Genetic variations in other appetite genes

Genetic variations in appetite genes have been identified in several species, some of which were found to significantly affect carcass characteristics in livestock species. In cattle, an exon 2 *C/T* SNP in *leptin* has been identified that causes a nonconservative aa substitution (Buchanan *et al*. 2002). The alleles of this SNP were found to significantly affect grade fat and average fat levels in beef cattle, where the *T/T* homozygotes had greater fat depth. This was later confirmed by Schenkel *et al*. (2005) as well as by Lusk (2007). The *T/T* homozygotes have also been suggested to be more likely to achieve AAA grading status (Kononoff *et al*. 2005). Genetic testing of *leptin* alleles is currently available to producers in Canada. This information is being used by the beef cattle industry as a tool for sorting animals at feedlot entry, as this can aid in the prediction of finishing time.

In swine, an exon 3 *C/T* SNP in *leptin* was shown to affect average daily gain as well as feed efficiency in Landrace and Yorkshire breeds (Chen *et al*. 2004). Further studies identified an exon 2 *C/T* SNP that significantly affected feed intake, average daily gain, and feed efficiency in Brazilian crossbred swine (de Oliveira Peixoto *et al*. 2006). This evidence suggests that genetic variation in leptin can lead to changes in the appetite and metabolic pathways in livestock species.

Mutations in the Melanocortin-4 Receptor (MC4R), the receptor for α-MSH and AGRP, have been associated with carcass traits in swine. A missense mutation at aa 298 has been identified, causing an Aspartic acid to be replaced by an Asparagine (Kim *et al.* 2000). The mutation was shown to have a significant effect on backfat, food intake, and growth rate in several pig lines. Variations have also been discovered in cattle MC4R. A *G/T* SNP has been identified which caused a Valine to Leucine substitution. This SNP was used to map bovine *MC4R* to BTA 24 (Thue *et al.* 2001).

Mutations in *AGRP* have been shown to affect macronutrient intake in humans (Loos *et al.* 2005). Two ethnic-specific polymorphisms were examined. A *C>T* SNP in the promoter sequence at position -38 was identified in African-Americans, and an *A/G* SNP in the third exon causing an Alanine to be replaced by a Threonine (Ala67Thr) was found in Caucasians (Loos *et al.* 2005). Both were shown to be significantly associated with energy intake. The promoter SNP was associated with protein intake in African-Americans, while the exonic SNP was associated with fat and carbohydrate intake in whites. It was postulated that differences in eating habits or genetic factors that influence taste and food preference may partially explain the observed differences between the two ethnic groups (Loos *et al.* 2005)

2.10 Relevance to the beef industry

The ideal beef carcass is one with just enough fat to produce tender and juicy cuts of meat but still contains a high percentage of lean meat. Many people enjoy a blue-rare steak while others prefer a well-done steak, indicating that consumer preferences vary widely. Producers and finishers are penalized for submitting carcasses that are

considered overweight or underweight, as processors like to receive animals that are roughly equivalent and contain a nice balance of marbling and lean meat. Profit margins are tight in the commercial beef industry and it is very obvious that over-finishing animals only cuts into that profit margin. Under-finished animals represent a situation where full profit potential has not yet been achieved. Genetically sorting cattle by *leptin* genotype allows producers to better predict how and when a particular animal will finish, producing more consistent carcasses. Genetic testing is also available for tenderness, marbling, and feed efficiency. These tools allow producers to maximize the genetic potential of all animals, widen their profit margins and make the beef industry more profitable, as well as to consistently produce superior beef. Cow-calf producers could also make use of this information to select the best animals for breeding and to make informed culling decisions.

3.0 HYPOTHESIS AND OBJECTIVES

The primary objective of this thesis was to sequence *Bos taurus Pro-Melanin-Concentrating Hormone*. The sequence obtained was to be used for characterization of this gene in cattle species, as well to assess the conservation of this gene with that of humans, mice, and rats. Genomic sequencing would also allow for the identification of allelic polymorphisms within the gene, which were to be analyzed for functional effects on cattle production traits pertaining fat production and carcass composition.

4.0 MATERIALS AND METHODS

4.1 Animal populations

The Canadian Beef Reference Herd (CBRH) was created using multiple ovulation and embryo transfer (MOET) and consisted of 17 full-sib families, 5 of which were purebred while the remainder were crossbred (Schmutz *et al*. 2001). The families ranged from 2 to 17 offspring, for a total of 136 calves. The 13 dams and 5 sires were purebred and included Charolais, Angus, Belgian Blue, Hereford, and Simmental breeds. Carcass and taste panel data were collected for a number of traits (Appendix A).

The Behaviour Population consisted of 382 feedlot steers used initially in a study of stress and behavioural parameters (Pugh 2007). These steers were purchased as commercial cattle in five lots from auction and therefore were predominantly crossbred. Behavioural measurements were applied equally to all animals, and all animals were on the same diet. The steers were slaughtered in October of 2006 and blue tag carcass data was collected (Appendix B).

An additional population of Purebred Bulls was used to determine allele frequencies in Simmental, Charolais, Angus, and Hereford cattle (Appendix C).

Genomic DNA from six *Bos indicus* sires was also obtained in order to identify potential genetic differences between *Bos indicus* and *Bos taurus PMCH*.

4.2 DNA extraction and cDNA synthesis

Genomic DNA extraction of semen from the bulls of the CBRH had previously been done as outlined by Schmutz *et al*. (1995) (Appendix D). Blood from both the CBRH and the Behaviour Population had been collected in EDTA and was extracted using a salt extraction method (Montgomery & Sise 1990) (Appendix D). Genomic DNA from the Purebred Bull population had been extracted using a phenol/chloroform method, as described by Fitzsimmons *et al.* (1998) (Appendix D). Genomic DNA extraction from semen of the *Bos indicus* animals followed the same methodology used to extract DNA from semen for animals in the CBRH (Appendix D).

Samples from assorted tissues were previously obtained from a 5 week-old Holstein calf, two Holstein-cross calves of less than 1 week old, and a 19 month-old crossbred *Bos taurus* steer. The tissue samples from the Holstein calf and the steer were immediately immersed in liquid nitrogen. Total RNA was extracted and cDNA was synthesized as described by Goodall & Schmutz (2007) (Appendix E). The tissue samples from the Holstein-cross calves were immediately placed in RNA*later*® (Ambion, Austin, TX) and frozen until subsequent RNA extraction at a later date. Synthesis of cDNA from total brain RNA of the steer, as well as from tissues obtained from the Holstein-cross calves was performed following a mixed protocol from Invitrogen (Burlington, Ont.) and Fermentas (Burlington, Ont.) (Appendix E).

4.3 Genomic DNA PCR reaction conditions

Polymerase Chain Reaction (PCR) primers (Table 4.1) were designed based on available human (NM_002674), mouse (NM_029971), and predicted bovine

(XM_584729) mRNA *PMCH* sequences, as well as from bovine genomic (NW_270499) sequence (NCBI 2005). Genomic DNA from five dams and five sires of the CBRH were used initially for Single Nucleotide Polymorphism (SNP) identification. Primers were designed to amplify the coding region, including the introns, of *PMCH* for an expected product of 1383 base pairs (bp). Additional primers were designed to amplify a portion of the upstream region of the gene for an expected product of 438 bp (Table 4.1). A schematic of *PMCH* primer binding locations are shown in Figure 4.1, while the location of primer binding sites in the DNA sequence are shown in Figure 4.2.

To amplify the coding region of *PMCH*, 25-50 ng of genomic DNA was added to each 14 μl reaction containing 0.2 mM dNTP, 2 mM $MgCl_2$, 10 pmol of each primer (*PMCH*for, *PMCH*rev; Table 4.1), 1X PCR buffer, 0.5 U of *Taq* Polymerase (Invitrogen), and 9.5 μl of deionized water (dH_2O). The reaction was carried out in a Stratagene® RoboCycler® PCR machine. Initial denaturation was 4 min at 95 °C, followed by 37 cycles of: denaturation for 50 s at 95 °C, annealing for 50 s at 53 °C, and extension at 72 °C for 1 min 30 s. The reaction concluded with a final extension for 4 min at 72 °C. The PCR reaction cocktail for upstream region amplification was the same as for the coding region reaction with the exception of the substitution of 3 mM $MgCl_2$, and 9.2 μl of dH_2O. The amplification cycle was also the same, with the exception of a 50 s extension time. The primers used were *Prom*For and *Exon1*rev (Table 4.1). PCR products were analyzed on a 2% agarose gel with a 1 kb plus DNA ladder (Gibco).

Table 4.1 Sequences of primers used for PCR and PCR-RFLP reactions.

Primer	**Sequence**
PMCH:	
*PMCH*for	GCAAACATCAAACTAAGGATGG
*PMCH*rev	CGTATGGTTAGCATGTTAAGC
*Prom*For	GGTTGGTTTCTATCTGATGAG
*Exon1*rev	GTCGCATTATCACTTACCTTTG
*cDNA*for	CAAAAATTCAAAGAATGCAGGTTCC
*cDNA*rev	GCATACACCTAAGCATGTCAAAATC
*BrEx1*for	CCTTGTTTGACTCTATGC
*BrEx3*rev	GACTTGCCAACAAGGTCG
*Mm*for	GATGAGTCATTTCTAAAATGACG
GAPDH:	
*GAPDH*for	GTTTGTGATGGGCGTGAAC
*GAPDH*rev	GTGGACAGTGGTCATAAGT

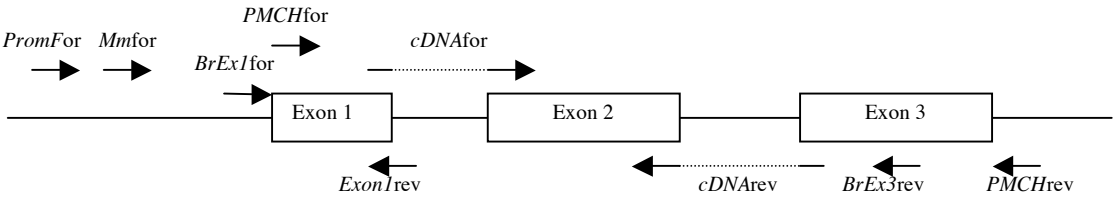

Figure 4.1 Schematic binding locations of *PMCH* primers. Forward primers are shown above and reverse primers are shown below the schematic *PMCH* gene. The hatching of primers *cDNA*for and *cDNA*rev indicates that these primers are exon-specific.

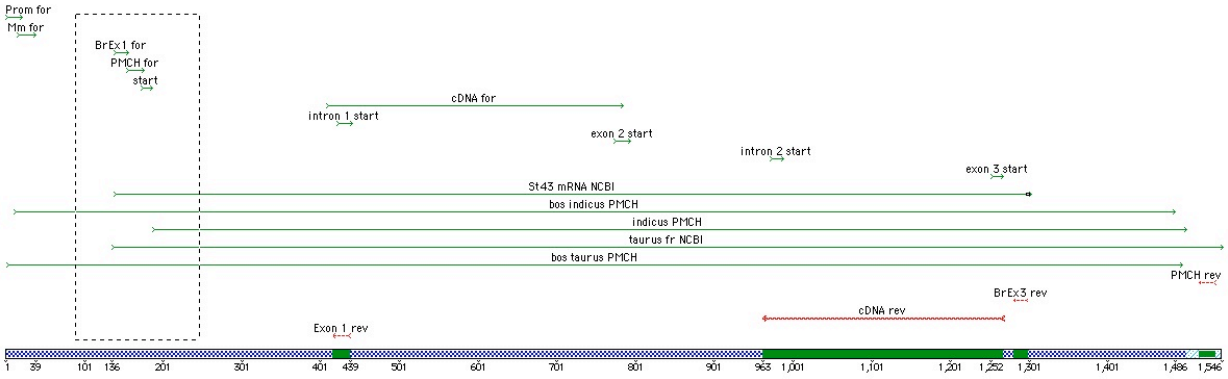

Figure 4.2 Location of primer binding sites in the *PMCH* DNA sequence. Above is a screenshot taken from Sequencher version 4.1 of the alignment of *PMCH*, both genomic and cDNA, with primers used in this study.

4.4 Sequencing

Genomic DNA from five of the dams (Angus_2, Charolais_9, Charolais_11, Belgian Blue_19, and Simmental_23) as well as all five sires (Angus_4, Limousin_8, Charolais_12, Hereford_16, and Simmental_24) of the CBRH, as well as genomic DNA from the six *Bos indicus* sires, were amplified by PCR and sent for DNA sequencing. PCR products were excised from the agarose gel and extracted following the protocol as outlined in the Gel Extraction Kit (Qiagen, Mississauga, Ont.). To quantify the products, 2 µl of each extracted product was then added to 5 µl of dH_2O and 1.5 µl of Ficoll Loading buffer and run alongside a DNA mass ladder (Gibco) in a 1.5% agarose gel. The extracted products were then sequenced on an Applied Biosystems Sequencer at the Plant Biotechnology Institute (Saskatoon, SK).

Brain cDNA from the 19 month-old crossbred steer was also chosen for sequencing. In order to obtain sequence from the entire coding region, two reactions were carried out as a result of the presence of spurious products. The amplification of exons 1 and 2 used primers *BrEx1*for and *cDNA*rev (Table 4.1). The amplification of exons 2 and 3 used primers *cDNA*for and *BrEx3*rev (Table 4.1). The conditions for both reactions were the same. The 15 µl reaction cocktail contained 5-8 ng of cDNA, 0.2 mM dNTP, 2.5 mM $MgCl_2$, 10 pmol of each primer, 1X PCR buffer, 0.5 U of *Taq* Polymerase (Fermentas), and 8.6 µl of dH_2O. PCR amplification began with an initial denaturation at 95 °C for 4 min, followed by 35 cycles of 95 °C for 50 s, annealing at 56 °C for 50 s, and extension at 72 °C for 50 s. Amplification concluded with a 4 min final

extension at 72 °C. Products were analyzed on a 1.5% agarose gel with a 1 kb plus DNA ladder (Gibco). Extraction, quantification, and sequencing of the brain cDNA product were the same as for genomic DNA.

4.5 PCR-Restriction Fragment Length Polymorphism

A PCR-Restriction Fragment Length Polymorphism (RFLP) assay was designed to genotype the remaining CBRH dams and offspring, as well as the Purebred Bull and Behaviour populations at the -134 SNP. In order to identify the SNP allele, a mismatch primer (*Mm*for) (Table 4.1) was designed to introduce a *TaiI* digest site in the presence of the thymine allele (Figure 4.3). The reaction used the reverse primer *Exon1*rev (Table 4.1). Digestion of the PCR product resulted in fragments of 21, 146, and 257 bp when the thymine allele was present, and fragments of 146 and 278 bp when the adenosine allele was present. Each 15 µl reaction cocktail contained 25-50 ng of genomic DNA, 0.2 mM dNTP, 2 mM $MgCl_2$, 10 pmol of each primer, 1X PCR buffer, 0.5 U of *Taq* Polymerase (Invitrogen), and 9.5 µl of dH_2O. The PCR reaction began with a 4 min denaturation at 95 °C, followed by 34 cycles of denaturation at 95 °C for 50 s, annealing at 53 °C for 50 s, and extension at 72 °C for 50 s, and finished with a 4 min final extension. 1 µl of *TaiI* and 1.5 µl of Buffer R (Fermentas) were then added to each 15 µl PCR reaction and digested at 65 °C for 2.5-3 hours. The digest was then analyzed on a 4% DNA agar gel (Marine Bioproducts, Delta, B.C.).

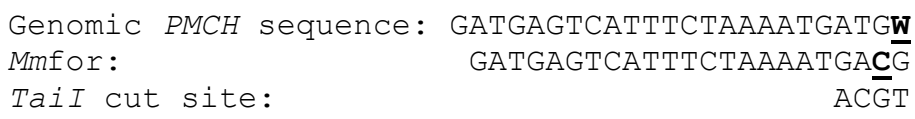

Figure 4.3 Mismatch primer design to introduce a *TaiI* restriction digest site in the presence of the thymine allele in the *Bos taurus* genomic *PMCH* sequence. The *A>T* SNP is shown as a W in the cattle genomic *PMCH* sequence. The mismatch was introduced at the 22nd nucleotide of the primer sequence to introduce a restriction digest site when the thymine allele was present and not when the adenosine allele was present. *TaiI* digests the DNA immediately following the thymine allele of the digest site.

4.6 Mapping

Once genotyping of the CBRH was completed, CRIMAP (Green *et al*. 1990) was used to analyze *PMCH* with previously genotyped microsatellites on BTA5 (Schmutz *et al*. 2001) (Appendix F).

4.7 Tissue expression profile

A tissue expression profile was obtained by performing PCR on cDNA samples from assorted tissues (spleen, adrenal gland, thymus, kidney, liver, lymph node, lung, heart, brain, spinal cord, skeletal muscle, adipose, rumen, abomassum, abomassal muscle, large intestine, and small intestine) obtained from the 5 week-old Holstein calf and the Holstein-cross calves, as well as the 19 month old steer. Primers *cDNA*for, *cDNA*rev were used (Table 4.1). Each 15 µl reaction contained 5-8 ng of cDNA, 0.2 mM dNTP, 2.5 mM $MgCl_2$, 10 pmol of each primer, 1X PCR buffer, 0.5 U of *Taq* Polymerase (Fermentas), and 8.6 µl of dH_2O. Amplification was carried out as follows: denaturation at 95 °C for 4 min, 37 cycles of denaturation at 95 °C for 50 s, annealing at 59 °C for 50 s, and extension at 72 °C for 50 s and concluded with a final extension of 72 °C for 4 min. Products were analyzed on a 1.5% agarose gel with a 1kb plus DNA ladder (Gibco). PCR for *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) was performed on all samples as a positive control, using the same PCR conditions. The reaction used primers *GAPDH*for and *GAPDH*rev (Table 4.1).

4.8 Statistical analysis of *PMCH* genotype on carcass traits

Statistical analysis of the *PMCH* genotype effect on carcass traits in both populations was carried out using SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC, USA). Carcass traits including taste panel evaluations, tenderness measurements, and fat measurements were examined in the CBRH, and fat measurements were examined in the Behaviour Population. The MIXED procedure was used to calculate a one-way analysis of variance (ANOVA) statistic and the least-squared means (LSM). The model used to determine the effect of *PMCH* genotype on trait was:

$$Y_{ij} = \mu + PMCH_i + e_{ij}$$

where Y_{ij} was the observation of the dependent variable (carcass trait) for the i^{th} animal, μ was the overall population mean of the dependent variable, $PMCH_i$ was the effect of genotype, and e_{ij} was the random error term for each experimental unit. A trait was considered to be significantly affected by *PMCH* genotype when $P \leq 0.05$ and trends declared when $0.05 \leq P \leq 0.1$. In the CBRH, gender was initially included in the model however no significant interaction between genotype and gender was found so gender was subsequently dropped from the model.

4.9 Transcriptional start site prediction

In order to predict the transcriptional start site of exon 1, the *Bos taurus PMCH* genomic sequence from Limousin 8 (accession number DQ499531, submitted) was analyzed using the Neural Network Promoter Prediction version 2.2 (1999) available online at http://fruitfly.org/seq_tools/promoter.html (Reese & Eeckman 1995) (Appendix G). The program identifies transcriptional elements within the submitted eukaryotic

promoter sequence and predicts the most likely transcriptional start site. Additionally, the cattle promoter region sequence obtained from sequencing was added to the alignment of human and rat *PMCH* promoter region sequence and transcriptional start site prediction as determined by Viale *et al*. (1997) for comparative purposes.

4.10 Computational analysis of the promoter region

A preliminary analysis of promoter binding sites was conducted by submitting the 5' sequences of each allele to P-Match, part of the Biobase Biological Database (2006), which can be found at www.gene-regulation.com. P-Match searches for potential transcription factor binding sites in any sequence using the TRANSFAC® database. The first 173 nucleotides of the sequenced *PMCH* 5' region from Charolais_12 (*A*/*A*) as well as Simmental_24 (*T*/*T*) were submitted. The matrix group chosen was vertebrates, with the cut-off set to minimize false negatives (Appendix H).

5.0 RESULTS

5.1 Sequencing and single-nucleotide polymorphism detection

Sequence data revealed the presence of an adenosine-to-thymine (*A*/*T*) SNP in the upstream region of *PMCH* at position -134 relative to the translation start codon in the heterozygous state in three of the *Bos taurus* animals sequenced (Figure 5.1). Three other animals were homozygous for the *T* allele, while the remaining four animals were homozygous for the *A* allele. Sequencing of the six *Bos indicus* sires revealed that five of them were *A*/*A*, while one was *A*/*T* at this SNP (Table 5.1).

A previously reported *A*/*C* SNP in *Bos indicus*/*Bos taurus* crossbred cattle (Stone *et al*. 2002) was observed in intron 2. In this study, one *Bos indicus* bull was *A*/*C* at this position, another was *C*/*T*, and the remaining four were homozygous *C*/*C* (Table 5.1). All *Bos taurus* animals were *A*/*A* at this SNP.

One *Bos indicus* sire had an additional *C*/*T* SNP in exon 1, 102 bp downstream of the translation start site, which did not alter the amino acid (Table 5.1). The five remaining *Bos indicus* sires were *C*/*C* at this position, whereas all *Bos taurus* animals sequenced were homozygous *T*/*T* (Table 5.1).

The complete genomic sequence from the sire Limousin_8 (heterozygote) of the CBRH was submitted to GenBank (accession number DQ499531) (Figure 5.1). The cDNA obtained from the brain of the crossbred steer was also sequenced and submitted

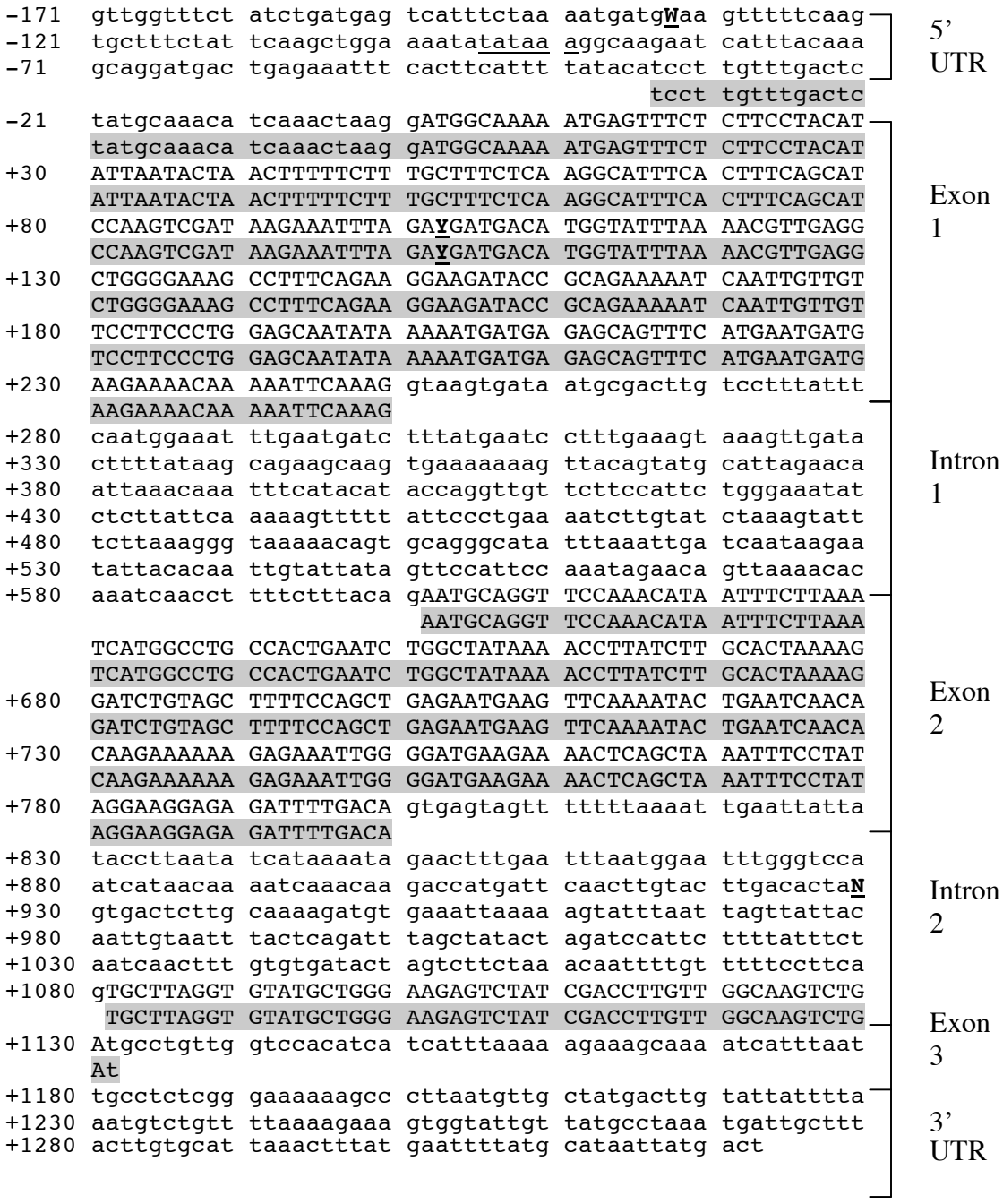

Figure 5.1 Alignment of sequenced *Bos taurus PMCH* genomic DNA (accession number DQ499531) and brain cDNA (accession number EF175214) from this study. Complementary DNA sequence is shaded. Lower case letters represent intronic sequence as well as 5’ and 3’ untranslated regions. The 5’ region *A>T* SNP at position -134 is represented by a W. The *C/T* SNP at position 102 in *Bos indicus* cattle is represented by a Y. The SNP in intron 2 is represented by an N. The putative TATA box is underlined.

Table 5.1 Haplotypes of the *Bos indicus* and *Bos taurus* animals at the three *PMCH* SNP identified in genomic sequence. Red alleles indicate a likely origin in *Bos taurus* cattle, while blue indicates a likely origin in *Bos indicus* cattle. The green allele is likely unique to *Bos indicus*.

Animal	-134 SNP	Exon 1 SNP	Intron 2 SNP
07-422 (*Bos indicus*)	*A/A*	*C/C*	***C/T***
07-423 (*Bos indicus*)	*A/A*	*C/C*	*C/C*
07-424 (*Bos indicus*)	*A/A*	*C/C*	*C/C*
07-425 (*Bos indicus*)	*A/**T***	***C/T***	***A/C***
07-650 (*Bos indicus*)	*A/A*	*C/C*	*C/C*
07-651 (*Bos indicus*)	*A/A*	***C/C***	***C/C***
Bos taurus	*A/A, A/**T**, **T/T***	***T/T***	***A/A***

to GenBank (accession number EF175214) (Figure 5.1). Genomic *PMCH* sequence from *Bos indicus* animal 07-424 (Table 5.1) was submitted to GenBank, as was animal 07-422 (Table 5.1) (Accession numbers EU101386 and EU113045, respectively). The remaining dams and all offspring of the CBRH, as well as the Purebred Bulls and Behaviour Population, were genotyped by a PCR-Restriction Fragment Length Polymorphism (RFLP) assay at the -134 *A>T* SNP (Figure 5.2). This SNP was chosen for further investigation because it was the only SNP present in *Bos taurus* animals.

The PCR-RFLP was designed to introduce a *TaiI* restriction digest site in the presence of the thymine allele and not in the presence of the adenosine allele. This resulted in thymine homozygous animals displaying digested alleles of 248 bp and 21 bp (not visible) on the electrophoretic gel (Figure 5.2). Adenosine homozygous animals displayed undigested alleles of 259 bp, while homozygous animals displayed one undigested allele of 259 bp, and digested alleles of 248 bp and 21 bp (not visible). The PCR fragment contained a naturally-occurring *TaiI* restriction digest site which resulted in the production of a 146 bp DNA fragment in all animals. This fragment is a desirable byproduct as its presence acts as a positive control to indicate that the restriction digest enzyme is active. The absence of this fragment would indicate that the restriction enzyme is not digesting the DNA.

Human *PMCH* cDNA was found to be 66% identical to cattle, mouse cDNA was 62% identical, and rat cDNA was 59% identical upon sequence comparisons. Translation of the cDNA sequence indicated that *Bos taurus* PMCH amino acid (aa) sequence is very similar to that of human, mouse, and rat. The cattle PMCH aa sequence was 92.0 % similar to that of human, 83.6 % similar to that of mouse, and 78.8 % similar

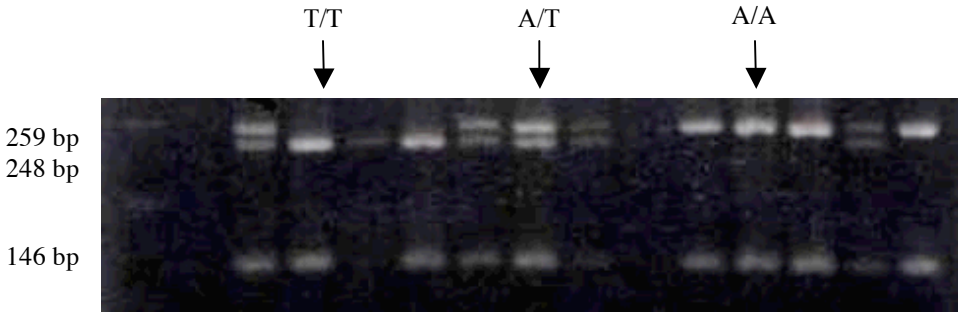

Figure 5.2 Agarose gel photograph showing the results of *PMCH* genotyping at the -134 SNP by *TaiI* PCR-RFLP. *T/T* animals have cut alleles of 248 bp, 146 bp, and a 21 bp band which is not visible. *A/A* animals have alleles of 259 bp and 146 bp. *A/T* animals have alleles of 259 bp, 248 bp, 146 bp, and 21 bp (not visible).

to that of rat (Figure 5.3). Cattle NGE was found to differ from human NGE by one aa at the 10th position. Mouse sequence had the same substitution at position 10 of NGE, as well as an additional aa substitution at position 13 that is distinct from human, rat, and cattle. Rat coding sequence had four aa substitutions at positions 2, 3, 4, and 10 of NGE. NEI and MCH aa sequence were perfectly conserved in all four species.

5.2 Allele frequency

The frequency of alleles for the -134 A>T SNP was determined in the CBRH and Behaviour Population because this is an indication of the potential usefulness of the SNP for genetic testing (Table 5.2). A breakdown of allele frequency by breed was determined using the Purebred Bulls (Table 5.2).

5.3 *PMCH* maps to BTA5

Cattle from the CBRH were genotyped at the -134 *A/T* SNP. Using multipoint linkage analysis of microsatellite alleles available from a previous study, and CRIMAP (Green *et al.* 1990), *PMCH* was linkage mapped on BTA5 (Figure 5.4). A log of the odds ratio (LOD) score of 3.23 was obtained between *PMCH* and *BL37*, of 4.85 with *MAF23*, and of 6.54 with *BMS1819*. LOD scores of >3.0 are considered significant and indicate that two markers or genes are genetically linked based on recombination frequencies between them. These findings are in agreement with those of another group who previously mapped *PMCH* to BTA5 using an intronic SNP (Stone *et al.* 2002).

Figure 5.3 PMCH amino acid alignment of *Bos taurus* (accession number EF175214), *Homo sapiens* (accession number NM_002674), *Mus musculus* (accession number NM_029971), and *Rattus norvegicus* (accession number NM_012625.1). NEI is indicated in blue and MCH is indicated in red. * indicates amino acid conservation of human, rat, or mouse with cattle PMCH sequence.

Table 5.2 *PMCH* -134 SNP allele frequencies in the CBRH and the Behaviour Population, as well as by breed in the Purebred Bulls.

Population	**n**	***A* frequency**	***T* frequency**
CBRH	122	0.68	0.32
Behaviour	382	0.67	0.33
Purebred Bulls:			
Angus bulls	55	0.83	0.17
Hereford bulls	21	0.64	0.36
Charolais bulls	52	0.73	0.27
Simmental bulls	18	0.42	0.58

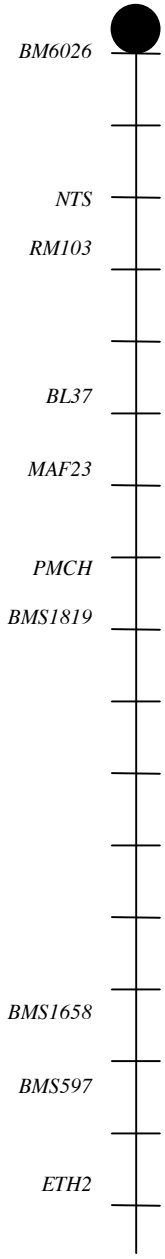

Figure 5.4 Depiction of *Bos taurus* chromosome 5 showing the experimental localization of *PMCH* and selected microsatellites based on CRIMAP linkage analysis (Green *et al.* 1990). Each line represents approximately 10 cM. *PMCH* was linkage mapped to approximately 71 cM from the centromere on BTA5.

5.4 Tissue expression profile

PMCH expression in various cattle tissues was assessed using cDNA-specific primers for PCR. *PMCH* expression was observed in most tissues examined including the spleen, heart, adrenal gland, lymph node, thymus, kidney, liver, brain, spinal cord, fat, rumen, abomassum, large intestine, abomassal muscle, and skeletal muscle (Figure 5.5). *PMCH* expression was not observed in the lung or small intestine. *GAPDH* expression was observed in all tissues examined.

5.5 Results of statistical analysis

5.5.1 CBRH population

Significant associations of *PMCH* were observed with carcass fat, as well as with shear force data and consumer taste panel evaluations. The Warner-Bratzler Shear Force value was developed by Bratzler in 1932 and is a quantitative measurement of the tenderness of cooked meat, where higher force measurements indicate tougher cuts of meat. The process involves drawing a flat blade through a specified size of cubed meat which rests in a triangular hole between two fixed plates. The amount of force necessary to draw the blade through the sample is the shear force score. The muscle fibers must run perpendicular to the blade path, and each sample is of a fixed cross-sectional area.

Palatability and tenderness are consumer taste panel traits that were collected for the CBRH at LaCombe, Alberta. Initial tenderness was evaluated as the perceived tenderness of the first bite, using the front incisors to bite perpendicularly through the

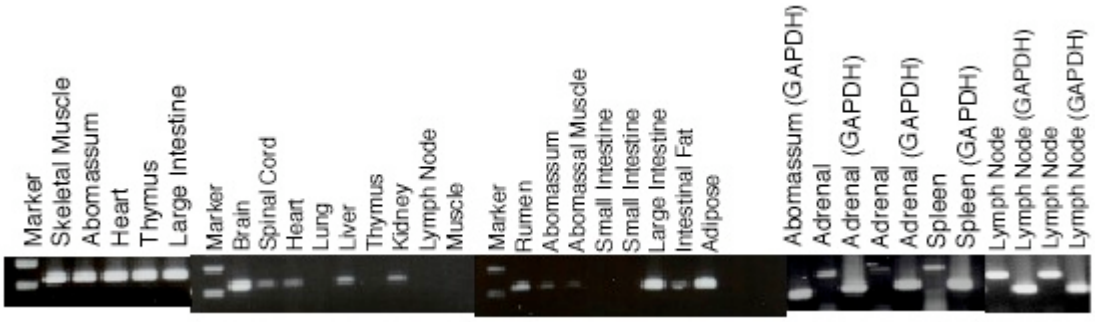

Figure 5.5 Agarose gel showing results of tissue expression analysis. All bands represent *PMCH* unless otherwise stated. The marker lanes show 200 and 300 bp markers from the 1kb ladder.

muscle grain. Overall tenderness was defined as the tenderness of the entire cube of meat, chewed with the molars just prior to being swallowed. The panelists were instructed to give numerical scores ranging from 1-9, with 1 being tough and 9 being tender. Palatability was defined as the overall experience of the meat, including flavor, juiciness, and tenderness and ranged from 1 (not palatable) to 9 (very palatable).

Carcass traits including average fat, grade fat, hip roast, shoulder roast, and steak shear force indicated significant associations ($P \leq 0.05$) or trends ($0.05 \leq P \leq 0.1$) with *PMCH* genotype in the CBRH (Table 5.3). Both average fat and grade fat showed an additive association with the *PMCH A* allele (Figure 5.6). Adenosine homozygotes also tended to have lower shear force values, indicating that cuts of meat from these animals were more tender than cuts from thymine homozygotes (Figure 5.7). The only exception was that of the hip roast measurement, where thymine homozygotes appeared to be more tender. Additionally, tenderness and palatability, as evaluated by a consumer taste panel, were found to have a significant association with *PMCH* genotype where the adenosine homozygotes were found to be most tender and palatable (Figure 5.8).

5.5.2 Behaviour Population

The Behaviour Population, chosen as typical of a feedlot population, also showed a significant effect of *PMCH* genotype on average fat and grade fat (Table 5.4). Both traits showed an additive relationship with *PMCH* genotype in which the adenosine

Table 5.3 Least Squared Means (LSM) and P-values of carcass traits significantly associated with *PMCH* genotype in the CBRH.

	ANOVA LSM [1]			
Trait	***T/T*** **n =20**	***A/T*** **n = 39**	***A/A*** **n = 63**	**P-value**
Average Fat (mm)	7.37 +/- 0.994 [a]	8.89 +/- 0.721 [ab]	10.29 +/- 0.564 [b]	0.031
Grade Fat (mm)	5.7 +/- 0.81 [a]	7.2 +/- 0.59 [ab]	8.2 +/- 0.46 [b]	0.024
Hip Roast [2]	6.45 +/- 0.278 [a]	7.12 +/- 0.199 [ab]	7.22 +/- 0.156 [b]	*0.056*
Shoulder Roast [2]	8.10 +/- 0.289 [ab]	8.31 +/- 0.207 [b]	7.38 +/- 0.163 [a]	0.001
Steak Shear Force (AB) [2, 3]	7.66 +/- 0.488 [ab]	7.68 +/- 0.341 [b]	6.41 +/-0.2668 [a]	0.006
Steak Shear Force (SK) [2, 4]	10.57 +/- 0.502 [b]	10.19 +/-0.360 [b]	8.05 +/- 0.283 [a]	<0.0001
Palatability [5]	4.16 +/- 0.220 [a]	4.50 +/- 0.158 [ab]	4.92 +/- 0.124 [b]	0.006
Tenderness (Initial) [5]	4.35 +/- 0.291 [a]	4.66 +/- 0.208 [ab]	5.22 +/- 0.164 [b]	0.016
Tenderness (Overall) [5]	4.26 +/- 0.295 [a]	4.61 +/- 0.211 [ab]	5.28 +/- 0.166 [b]	0.004

[1] Means with the same letter are not significantly different (P>0.05).
[2] Warner-Bratzler shear Force measurements.
[3] The steaks were cooked to well-done at this testing facility.
[4] The steaks were cooked to medium at this testing facility.
[5] These traits were evaluated by a consumer taste panel.

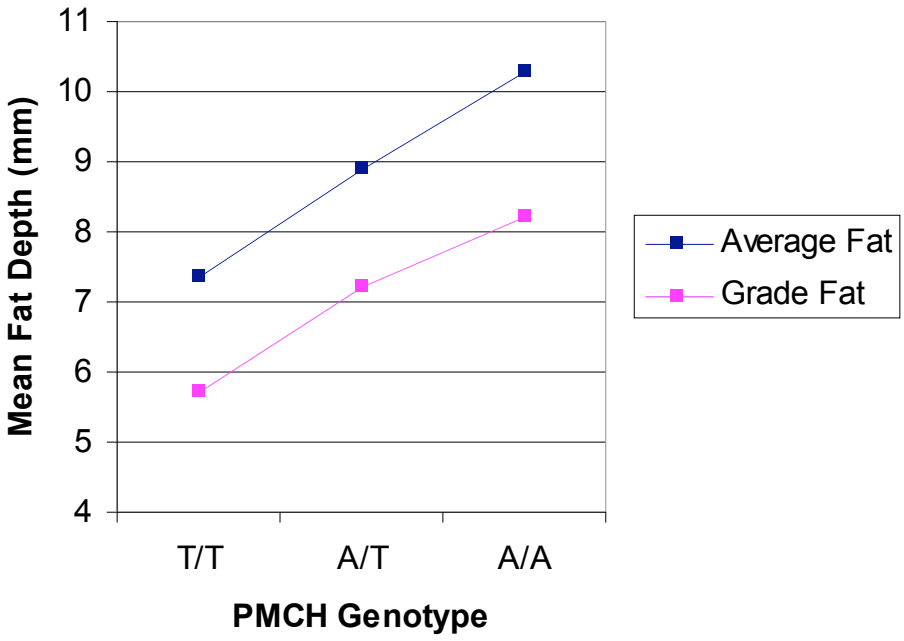

Figure 5.6 Graphical depiction of results from statistical analysis of *PMCH* genotype on average fat and grade fat in the CBRH.

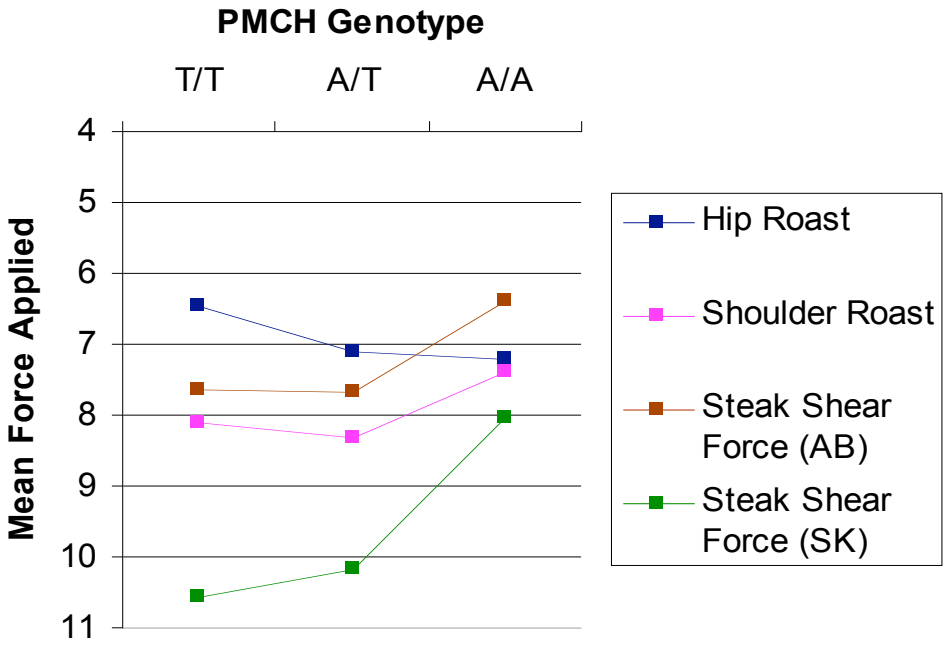

Figure 5.7 Graphical depiction of results from statistical analysis of *PMCH* genotype on Warner-Bratzler shear force measurements in the CBRH. Steak shear force values were obtained in Alberta and Saskatchewan.

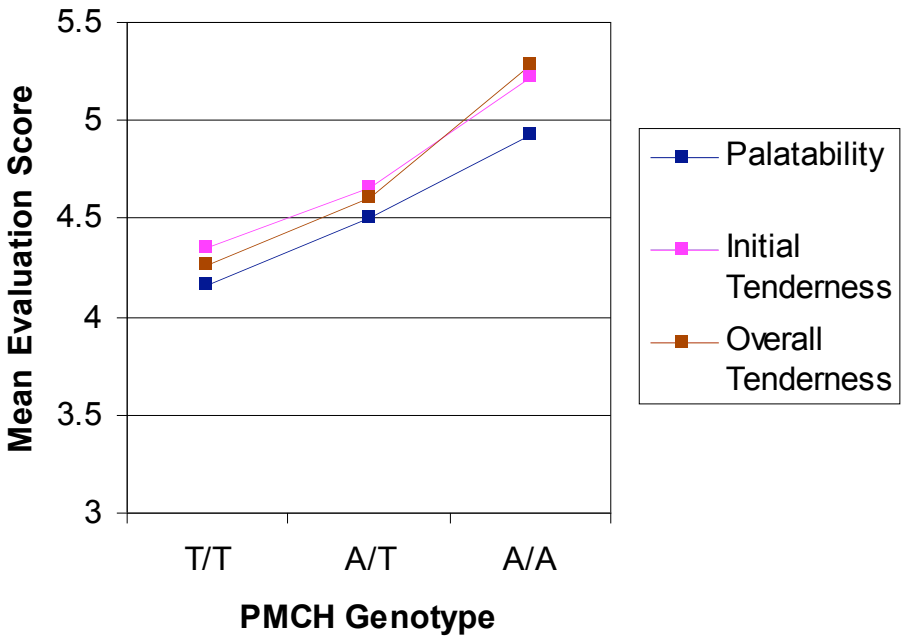

Figure 5.8 Graphical depiction of results from statistical analysis of *PMCH* genotype on consumer taste panel evaluations. The evaluations were based on a scoring system where 1 = most tough and 9 = most tender.

Table 5.4 Least Squared Means (LSM) and P-value for carcass traits significantly associated with *PMCH* genotype in the Behaviour Population.

	ANOVA LSM*			
Trait	***T/T*** **n = 47**	***A/T*** **n = 155**	***A/A*** **n = 180**	**P-value**
Average Fat (mm)	8.62 +/- 0.530 [a]	9.70 +/- 0.292 [ab]	10.09 +/- 0.272 [b]	0.047
Grade Fat (mm)	7.2 +/- 0.53 [a]	8.3 +/- 0.29 [ab]	8.6 +/- 0.27 [b]	0.046

*Means with the same letter are not significantly different (P>0.05).

homozygotes had the highest levels of subcutaneous fat deposits (Figure 5.9). Meat quality traits including shear force values and consumer taste panel evaluations were not available for this population.

5.6 *PMCH* transcription start site and promoter region analysis

The results of transcription start site prediction analysis by the Neural Network Promoter Prediction version 2.2 algorithm (NNPP) on genomic sequence obtained from Limousin_8 (accession number DQ499531, submitted) provided four possible transcription start sites. The first prediction concerning the transcriptional start site was at nucleotide 103 of the submitted sequence, which would place the transcriptional start site 28 bp downstream of the predicted TATA Box (Figure 5.1). Based on the comparison of this predicted start site with the other three predictions made by NNPP, it appeared as though this prediction was the most likely to be correct. The three other predictions indicated the start sites at position 317, 399, and 689 nucleotides of the submitted cattle sequence respectively, which would exclude portions of the known coding sequence. Nucleotide 317 is in exon 1, as is nucleotide 399. Nucleotide 689 is in intron 2. If transcription were to initiate at these predicted sites, the result would contradict the *PMCH* cDNA sequence obtained by omitting part or all of exon 1. The first prediction by the NNPP software also agreed with the transcription start site prediction made by others concerning the rat and human (Viale *et al*. 1997). The use of TATA box sequences and the exon 1 start site in the human and rat *PMCH* sequences as

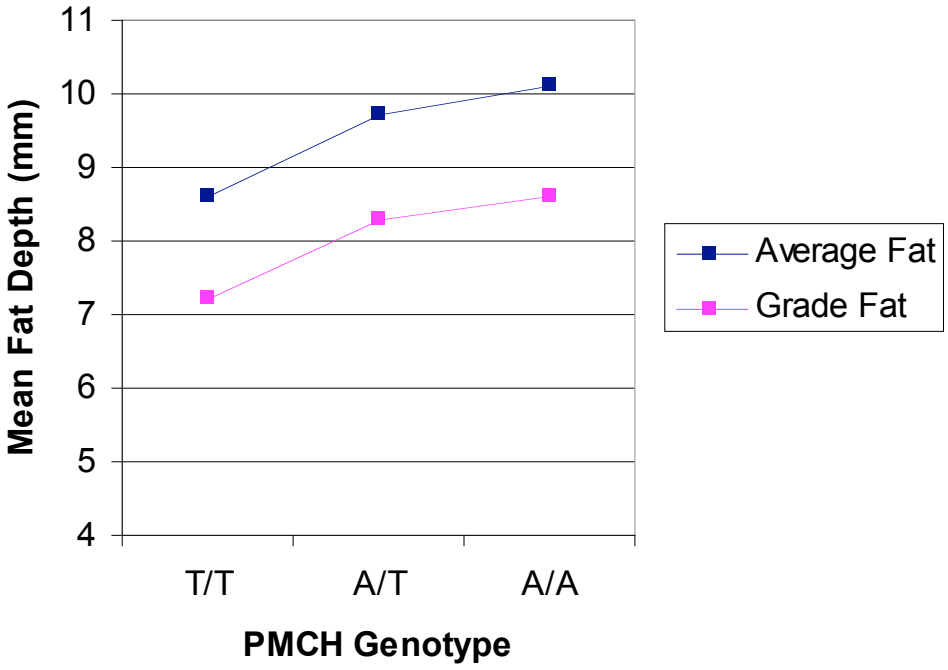

Figure 5.9 Graphical depiction of results from statistical analysis of *PMCH* genotype on average fat and grade fat in the Behaviour Population.

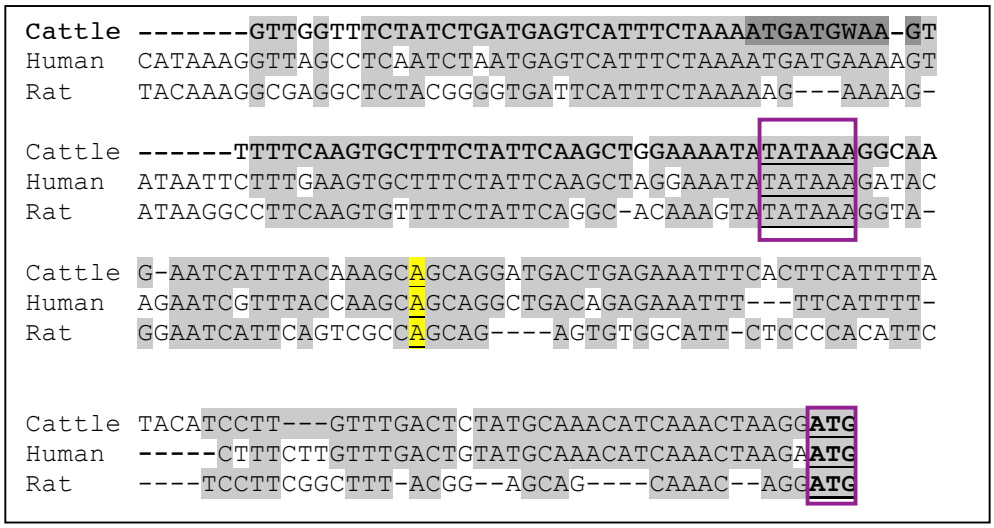

Figure 5.10 Alignment of the promoter regions from cattle, human, and rat *PMCH*. Shaded areas represent conservation with cattle sequence. The -134 *A>T* SNP in cattle is indicated by a W. The putative TATA box and the translation start site are boxed. The putative transcription start site is highlighted in yellow. The putative E4BP4 binding site in cattle is shown in dark grey. (Cattle sequence is accession number DQ499531, submitted; Rat sequence is accession number NC_005106.2; Human sequence and transcription start site predictions in human and rat were adapted from Viale *et al*. 1997)

anchors, as well as the output from NNPP, allowed the start site of *Bos taurus* to be putatively placed 103 bp downstream from the beginning of the sequenced cattle gene, 28 bp downstream of the predicted TATA box (Figure 5.10).

The results of P-match analysis on the cattle *PMCH* promoter region indicated the presence of a binding site for a transcriptional repressor, Adenovirus E4 promoter binding protein (E4BP4), in the presence of the thymine allele (Simmental_24) on the (+) DNA strand (Appendix H). The core similarity score, representing the similarity of the submitted sequence to the five most conserved nucleotides in the consensus transcription factor binding site, was a perfect 1.0. The matrix score, representing overall similarity between the consensus transcription factor binding site and the submitted sequence was 0.993. This binding site was not identified by P-match in the presence of the adenosine allele (Charolais_12). The consensus binding site for E4BP4 is (*G*/*A*)*T*(*G*/*T*)*A*(*C*/*T*)*GTAA*(*C*/*T*) (Cowell *et al.* 1992). The *Bos taurus PMCH* SNP is located at the 7th position of this consensus sequence. (Figure 5.10; Figure 5.11), which matches the E4BP4 consensus binding site with the exception of the presence of a guanine at the 10th position instead of a cytosine or thymine. A sequence matching the E4BP4 consensus sequence appears to be absent in both rats and humans as they both contain an adenosine residue at position 7 (Figure 5.11). Human sequence also contains an inserted adenosine at the 10th position within the putative E4BP4 consensus sequence. Rat sequence bears very little similarity to the putative E4BP4 consensus sequence as rats have additional non-consensus residues at positions 2, 5, 6, and 8 (Figure 5.10; Figure 5.11).

```
E4BP4 consensus sequence:(G/A)T(G/T)A(C/T)GTAA(C/T)
Cattle sequence:                ATGATG**T**AAg
Human sequence:                 ATGATGaAAa
Rat sequence:                   AaGAaaagAT
```

Figure 5.11 Analysis of potential E4BP4 binding sites in genomic *PMCH* sequence from cattle, human, and rat. Consensus nucleotides are in uppercase while non-consensus nucleotides are in lower case. The location of the SNP (thymine allele is shown) is bolded.

6.0 DISCUSSION

6.1 Haplotype analysis

Examination of the six *Bos indicus* haplotypes available (Table 5.1) indicated that bull 07-425 may be crossbred. This animal was homozygous at the -134 SNP, while the five other *Bos indicus* animals had only adenosine alleles. Additionally, the exon 1 *C>T* SNP may be a reflection of this. The *Bos indicus* animals sequenced, with the exception of this bull, had only the cytosine allele at this position, while all *Bos taurus* animals had only the thymine allele. Animal 07-425 was heterozygous *C/T* at this position. Initially, there appeared to be three alleles at the intron 2 SNP in the *Bos indicus* animals, which was initially reported as an *A/C* SNP in *Bos indicus*/*Bos taurus* crossed cattle (Stone *et al.* 2002). Closer inspection of the alleles at this position also supported the hypothesis that animal 07-425 may be crossbred. Four of the *Bos indicus* sires sequenced in this study were homozygous *C/C* at this position while another was *C/T*, whereas animal 07-425 was heterozygous *A/C*, consistent with the Stone group's findings. All *Bos taurus* animals sequenced were homozygous *A/A* at the intron 2 SNP. These data suggest that the adenosine allele of the intronic SNP originates in *Bos taurus* cattle, while *Bos indicus* cattle appear to carry a cytosine allele. The presence of the thymine allele at this position in one *Bos indicus* animal may indicate that this allele is a genuine SNP present exclusively in *Bos indicus* cattle.

Although the thymine allele at position -134 was not observed in the five *Bos indicus* animals that appear to be purebred, with this small sample size we cannot exclude the possibility that the thymine allele exists at this position in purebred *Bos indicus* animals. The apparent discrepancy between these taxonomic groups may simply be a result of the limited number of *Bos indicus* animals sequenced, exaggerated by a low representation of the minor allele in these animals by chance.

Based on the results of the haplotype analysis, the SNP alleles can be tentatively assigned to *Bos taurus* or *Bos indicus* cattle. It appears that the adenosine allele at the -134 *A>T* SNP is present in both taxonomic groups, while the thymine allele is predominantly present in *Bos taurus* cattle. The exon 1 *C>T* SNP could potentially be used to differentiate *Bos indicus* from *Bos taurus* as *Bos indicus* animals appear to have only cytosine, while *Bos taurus* animals appear to have only thymine. The adenosine allele at the intron 2 SNP appears to be present only in *Bos taurus* cattle, while *Bos indicus* cattle have a cytosine or a thymine at this position. These findings could potentially be used as part of a SNP-based test to determine whether an animal is *Bos taurus*, *Bos indicus*, or crossbred. Further assessment of *Bos indicus PMCH* sequence should be undertaken to ensure the validity of these assumptions before such a test is developed.

6.2 Allele frequencies

Allele frequencies of the -134 *A>T* SNP in the CBRH and the Behaviour Population (Table 5.2) indicated that the adenosine allele is most prevalent. Allele frequencies in the Purebred Bulls (Table 5.2) indicated that Simmental cattle tended to

have the lowest frequency of the adenosine allele. Continental breeds, including Charolais and Simmental, are thought to be leaner and more heavily muscled than their British (Angus and Hereford) counterparts, due to the earlier maturation of British cattle (Gregory *et al*. 1994; Wheeler *et al*. 2005). Angus cattle had the highest frequency of the adenosine allele, while Simmental animals had a higher frequency of the thymine allele. These data indicate that the thymine allele is present in cattle populations often enough to potentially limit feedlot performance, making a genetic test valuable to producers.

In addition to identifying three SNP, sequencing also revealed the use of a less common donor splice site at the end of exon 2. The donor splice site at the 3' end of exon 2 in cattle differs from the most common *AG*/*gt* by substituting *CA*/*gt* (Figure 5.1). Nucleotide substitutions at this site have been previously observed. Research has indicated that the conservation of the exonic adenosine allele at this position is approximately 60 %, while conservation of the exonic guanine allele is approximately 79 % (Jackson 1991; Cartegni *et al*. 2002). The same variant donor site was observed at the end of exon 2 in both human (Breton *et al*. 1993b; Viale *et al*. 1997) and mouse (Breton *et al*. 1993a) *PMCH* sequences.

6.3 Mapping and quantitative trait loci

PMCH was placed on BTA5 by linkage mapping in the CBRH (Figure 5.4). Other groups have identified quantitative trait loci (QTL) for backfat on BTA5. A significant association with backfat in the approximate region of 62-72 cM on BTA5 in Piedmontese/Belgian Blue crosses has been identified (Casas *et al*. 2000). Additionally, QTL for backfat on BTA5 in the approximate region of 65.4-70 cM has been identified

using haplotype analysis in a commercial line of *Bos taurus* (Li *et al*. 2004). The statistical association of *PMCH* alleles with average fat and grade fat (Tables 5.3 and 5.4), as well as linkage mapping results, are consistent with these findings.

6.4 Tissue expression profile

PMCH expression has been previously reported in the intestine of rats, humans, and mice, and additionally in the stomach of mice and rats but not humans (Viale *et al*. 1997; Hervieu & Nahon 1995; Breton *et al* 1993a). Analysis of the cattle small intestine revealed that *PMCH* is not expressed in this tissue, but was noted in the cattle rumen and abomassum, as well as the large intestine (Figure 5.5). A ruminant's abomassum is similar to a monogastric stomach therefore one would expect *PMCH* to be expressed in the abomassum (Leek 1993). The cattle rumen is not a secretory stomach (Leek 1993), indicating that the mechanism of *PMCH* action may be slightly different in ruminants.

PMCH expression was additionally found in a variety of tissues, indicating a widespread expression profile in the calf (Figure 5.5). The role of *PMCH* in the majority of these tissues is unknown. *PMCH* mRNA is expected to be present in the brain, where it can participate as an effector in the appetite pathway and interact with other peptides such as leptin. *PMCH* may be responsive to immune status as suggested by the observed presence of mRNA in the lymph nodes, thymus, and spleen (Dickson 1993; Swenson 1993), and may possibly function to provide the link between disease status and food intake. Expression of *PMCH* in these tissues has previously been confirmed in humans (Viale *et al*. 1997), while expression in the spleen has been confirmed in mice (Breton *et al*. 1993b). The presence of *PMCH* in the kidneys may be as a result of a functional

response to the concentration of urine produced in order to aid in the maintenance of water homeostasis (Reece 1993). *PMCH* mRNA was not, however, found in the kidneys of humans (Viale *et al*. 1997). *PMCH* was additionally detected in the cattle heart. *PMCH* mRNA has been detected in the mouse heart but not in the human heart (Breton *et al*. 1993a; Viale *et al*. 1997). The role of *PMCH* in the heart is unknown.

The presence of *PMCH* mRNA in the calf adrenal gland (Figure 5.5) may enable a response to the production of adrenal hormones such as cortisol. Glucocorticoids from the adrenal gland act on adipose tissue and skeletal muscle to regulate the rate of fat breakdown, as well as on immune tissues to produce an anti-inflammatory response (Dickson 1993). Adrenalectomy in rats, resulting in the removal of glucocorticoids, has been shown to attenuate the effect of MCH on food intake, suggesting that glucocorticoids are essential to potentiate MCH (Drazen *et al*. 2004). *PMCH* expression was additionally noted in adipose tissue and muscle samples including abomassal and skeletal (Figure 5.5), which supports this notion. *PMCH* mRNA has also been reported in adipose tissue of rats (Bradley *et al*. 2000) and humans, as well as in human adrenal gland (Viale *et al*. 1997).

6.5 Statistical analysis

Average fat and grade fat were significantly affected by *PMCH* genotype in the CBRH (Table 5.3) and the Behaviour Population (Table 5.4). Both fat traits showed an additive relationship with *PMCH* genotype which favored the *A* allele (Figure 5.6, Figure 5.9).

Shear force values were available for the CBRH animals only and were not validated in other populations. The values obtained from shear force measurements of adjacent tenderloin steaks (done both in Alberta and Saskatchewan), cooked to different temperatures, indicated that tenderness was increased in the *A/A* animals over the *T/T* animals (Table 5.3, Figure 5.7). In Alberta, the steaks were cooked to well-done, while in Saskatchewan the steaks were cooked to medium. The Alberta measurements were generally lower than those obtained in Saskatchewan, which may be a result of different cooking temperatures, or may also be a reflection of differing machine calibration or blade sharpness. The Alberta measurement for shear force of hip roast decreased in tenderness between *T/T* and *A/A* animals, but was only a trend (Figure 5.7). The shear force measurement of shoulder roast in Saskatchewan agreed with other statistical findings and was most tender when the animal was *A/A* (Figure 5.7). The shear force values for roasts were taken on different cuts of meat, which may, in part, explain the observed differences between roast shear force values obtained in Alberta and Saskatchewan.

Both initial and overall tenderness, as well as palatability, were found to be significantly affected by *PMCH* genotype in the CBRH (Table 5.3). The data obtained indicated that panelists found steak from *A/A* animals more palatable than steak from *T/T* animals (Figure 5.8). The panelists also indicated that they found *A/A* beef more tender, both initially and overall (Table 5.3, Figure 5.8). These findings are in agreement with the shear force data obtained and support the hypothesis that the adenosine allele contributes to the tenderness and overall palatability of beef.

6.6 Transcriptional start site

The cattle *PMCH* exon 1 start site was predicted to be located 103 bp downstream from the beginning of the cattle sequence obtained (accession number DQ499531, submitted) and 28 bp downstream from the predicted TATA Box as a result of NNPP analysis. In support of this result, it has been determined that there are generally 25-30 nucleotides between the TATA Box and the transcriptional start site in RNA Polymerase-II transcribed genes (Smale & Kadonaga 2003).

The alignment of the promoter region of *PMCH* of cattle with that from rat, mouse, and human indicates that the thymine allele is most likely to be the variant allele in cattle, as an adenosine is present at this position in rats, mice, and humans (Figure 5.10). The relative abundance of the adenosine allele in both *Bos taurus* and *Bos indicus* cattle (Table 5.1) is also supportive of this theory.

6.7 SNP function

Due to the location of the SNP it was postulated that it may affect transcription rates of *PMCH*. As a result of the P-match analysis, a binding site for a transcriptional repressor, Adenovirus E4 promoter binding protein (E4BP4), was discovered overlapping the SNP in the presence of the thymine allele which was absent in the presence of the adenosine allele (Figure 5.10). The similarity scores, both matrix and core, for the sequence submitted and the consensus binding sequence of E4BP4 indicated a very high degree of similarity, virtually eliminating the possibility that this was a false positive result. The cattle *PMCH* SNP is located at the 7th residue of the E4BP4 consensus

sequence, which appears to always be a thymine. The substitution of a thymine for an adenosine in the cattle *PMCH* 5' region may create an E4BP4 binding site, which may in turn affect gene expression levels.

The substitution of a guanine for a cytosine or thymine at the 3' end of the consensus binding sequence is not expected to interfere with E4BP4 binding. E4BP4 has been shown to bind to and decrease transcription rates when this non-consensus nucleotide is present (Lai & Ting 1999). The binding site analyzed had two additional non-consensus nucleotides at the 1st and 6th positions (Lai & Ting 1999) which indicates that E4BP4 is tolerant of certain non-consensus nucleotides within the consensus binding site and may also indicate that E4BP4 is able to bind the cattle sequence when the adenosine allele is present.

E4BP4 is a member of the basic region leucine zipper protein (bZIP) family of transcriptional repressors and is ubiquitously expressed (Cowell *et al*. 1992). E4BP4 is thought to play a role in the regulation of the mammalian circadian oscillatory mechanism, which is responsible for adaptations to daily environmental changes (Cowell 2002). *E4BP4* mRNA levels have been observed to increase in response to glucocorticoids, and binding sites for E4BP4 have been observed in genes that are negatively regulated by glucocorticoids. There is also evidence suggesting that E4BP4 activity is oscillatory, exhibiting maximal repressor activity during the evening and minimal repressor activity during the day (Mitsui *et al*. 2001). Evidence has suggested that E4BP4 most likely functions by interacting with the general transcription machinery,

by destabilizing or inhibiting the formation of the pre-initiation complex (PIC). This mechanism would allow E4BP4 to repress both basal and activated transcription (Cowell *et al*. 1992; Cowell & Hurst 1994).

One possible explanation for the potential mechanism of the SNP is that, due to the introduction of the thymine allele, an E4BP4 binding site is created which may be responsible for decreased transcription of *PMCH*. The high frequency of the adenosine allele observed in cattle populations and the presence of this allele in both humans and rats suggests that this allele is likely the original allele and the thymine allele is likely to be the variant allele. It is therefore unlikely that the introduction of the adenosine allele is relieving the gene of negative regulation by E4BP4 due to disruption of the consensus binding site. The mechanistic model may involve the identification of partial glucocorticoid response elements (GREs) in the promoter region of human and rat *PMCH* (Viale *et al*. 1999), which suggests that glucocorticoids may induce transcription of *PMCH* in these species. If these GREs are actively regulating *PMCH* expression, it is possible that the induction of E4BP4 by glucocorticoids mistakenly inhibits *PMCH* transcription in the presence of the thymine allele but not in the presence of the adenosine allele (Figure 6.1). On the contrary, the presence of glucocorticoids would not repress transcription of *PMCH* in the presence of the adenosine allele, where the binding site for E4BP4 does not exist. The observation that thymine homozygous animals had less average fat and grade fat than adenosine homozygotes supports the hypothesis that decreased transcription of *PMCH* in the presence of the thymine allele is the means by which this SNP exerts a functional effect. These suggestions are by no means conclusive, but provide a possible mechanism for the action of the SNP.

Figure 6.1 The potential mechanism of *PMCH* SNP action. In the presence of glucocorticoids, E4BP4 expression is upregulated. E4BP4 then binds to the promoter region of genes with E4BP4 binding sites. A) In the presence of the thymine allele, E4BP4 successfully binds and represses transcription of *Bos taurus PMCH*. B) In the presence of the adenosine allele, E4BP4 fails to recognize the binding sequence and does not bind, therefore transcription of *Bos taurus PMCH* is not repressed. The SNP alleles are shown in uppercase.

Promoter mutations have been identified in other species and have been associated with genetic dysregulation. Screening of 197 genes from the human genome for promoter SNPs revealed that approximately 23% of SNPs were predicted to cause the loss of a transcription factor binding site, while approximately the same number were predicted to introduce a new binding site (Sinnett *et al.* 2006). The remaining SNPs were predicted to result in a combination of losses and gains. It was suggested that allelic imbalances, due to the unequal expression of two alleles, may be attributable to these promoter SNPs. Mutations in the Prion Protein gene (*PRNP*) have been associated with susceptibility to prion infection in humans, sheep, and cattle (Sander *et al.* 2005). In cattle an insertion/deletion (indel) mutation in the promoter region as well as another in exon 1 were found to be associated with increased susceptibility to Bovine Spongiform Encephalopathy (BSE). Animals carrying both deletions were found to have increased expression of *PRNP* and showed increased susceptibility to BSE infection (Sander *et al.* 2005). These findings are supportive of the theory that genetic variations in gene promoter regions can contribute to alterations in gene regulation.

6.8 Implications

Given the preponderance of the adenosine allele compared to that of the thymine allele at the -134 SNP in the Canadian cattle populations tested, it appears as though cattle breeders have selected for cattle with the adenosine allele. This is likely because this allele was associated with visual traits they found desirable. These observations support the statistical findings that adenosine homozygotes have the highest levels of backfat, as well as being most tender and palatable. Despite this possibility, there remain

a significant proportion of cattle with the thymine allele (~30%) which may contribute to the limitation of performance and consistency of feedlot cattle. The cattle industry could make use of these findings to genetically select for *A/A* cattle, and to sort feedlot animals by genotype in order to finish them faster and produce more tender cuts of meat.

7.0 CONCLUSIONS

An upstream region *A>T* SNP at position -134 relative to the translation start codon in *Bos taurus PMCH* has been identified and the alleles of this SNP were shown to be significantly associated with average fat and grade fat levels in *Bos taurus* cattle. Animals homozygous for the adenosine allele had significantly more backfat on average then animals homozygous for the thymine allele, indicating that the adenosine allele additively favors backfat production. The SNP may also influence tenderness and palatability, as indicated by taste panel evaluations and Warner-Bratzler shear force measurements. Cuts of meat from adenosine homozygotes were indicated to be more tender and palatable than those from thymine homozygotes.

As a result of P-match analysis of transcription factor binding sites, it is believed that this SNP is introducing a novel transcriptional repressor binding site for E4BP4 in the presence of the thymine allele. This is believed to reduce *PMCH* gene expression in the presence of the thymine allele, which would be expected to reduce feed intake and possibly decrease the amount of fat produced by the animal. By altering the level of transcription, the SNP may be affecting the amount of functional peptide available, which would affect the balance of feed intake and metabolism in cattle.

Genetic variations in appetite-related genes such as *PMCH* have the potential to produce profound effects on carcass composition in cattle by altering the balance of feed intake and metabolism. Allelic variation in genes involved in appetite and metabolic

regulation may allow cattle producers to selectively breed and sort cattle, allowing the compilation of uniform cattle groups that will finish consistently in a predictable time frame with an ideal balance of fat and lean muscle. The ability of producers to breed and sort cattle by genotype could potentially have large economic consequences to the feedlot industry.

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9.0 APPENDICES

Appendix A Canadian Beef Reference Herd *PMCH* genotypes and trait values

Family	Tag	Sex	*PMCH*	Hip Roast*	Shoulder Roast*	Steak Shear Force SK*	Steak Shear Force AB*	Average Fat
3	25	M	*A/A*	8.01	8.01	9.84	6.44	6.67
3	27	F	*A/A*	7.04	6.69	7.30	7.32	13.00
3	28	F	*A/A*	6.50	7.15	5.82	5.41	12.33
3	29	M	*A/A*	6.87	8.86	9.16	7.33	11.00
3	33	M	*A/A*	8.29	6.61	9.51	6.99	6.67
3	34	M	*A/A*	8.20	7.73	7.01	7.34	9.00
4	35	M	*A/A*	10.36	10.14	10.43	5.56	6.33
4	36	F	*A/A*	6.99	6.19	6.83	3.63	12.00
10	46	F	*A/A*	6.75	6.38	6.16	4.67	9.00
4	48	M	*A/T*	5.16	7.36	8.83	4.94	9.33
4	57	F	*A/A*	7.01	8.31	9.23	5.22	15.33
6	61	M	*A/T*	7.60	9.34	14.29	7.83	8.67
12	65	F	*A/T*	5.60	8.96	10.10	4.98	14.67
9	69	F	*A/A*	6.87	7.71	9.29	4.97	12.00
8	74	F	*T/T*	6.04	7.27	9.77	6.39	12.00
6	76	M	*T/T*	6.40	9.52	11.21	5.43	7.67
5	84	M	*A/T*	7.34	8.15	17.15	13.23	12.00
6	86	M	*T/T*	7.88	9.72	14.47	9.20	4.00
6	87	M	*T/T*	7.34	10.57	13.19	12.73	5.00
8	92	M	*T/T*	6.97	8.85	15.62	10.14	5.33
6	93	M	*A/T*	9.89	8.59	16.17	11.25	8.00
7	94	M	*A/T*	5.86	7.22	9.14	5.70	8.00
7	98	M	*A/A*	8.02	7.80	12.26	9.40	3.33
6	100	F	*T/T*	6.07	7.29	10.88	8.21	8.67
9	103	F	*A/T*	9.15	9.25	10.57	7.34	6.33
2	104	F	*A/A*	6.12	5.57	5.87	4.47	16.33
2	107	M	*A/A*	6.47	6.28	9.42	7.82	7.33
9	108	F	*A/T*	8.48	8.09	8.32	6.56	9.67
9	109	F	*A/T*	6.40	6.57	11.14	8.47	6.67
7	110	M	*A/T*	7.28	8.42	8.91	6.32	3.67
10	112	F	*A/T*	7.17	9.44	5.91	5.53	11.67
9	113	M	*A/T*	6.65	7.00	11.25	7.76	4.00
7	114	M	*A/T*	7.28	10.99	11.00	7.26	7.33
8	115	F	*T/T*	5.42	7.73	10.95	6.47	18.67

* These are Warner-Bratzler Shear Force measurements.

Family	Tag	Sex	*PMCH*	Hip Roast	Shoulder Roast	Steak Shear Force SK	Steak Shear Force AB	Average Fat
7	116	F	*A/A*	5.91	8.27	6.34	4.97	10.67
7	119	F	*A/A*	7.65	6.12	6.64	5.72	12.00
7	122	M	*A/T*	9.16	9.28	10.45	8.27	10.00
11	124	M	*A/T*	6.00	10.71	9.54	6.51	1.67
2	126	F	*A/A*	6.97	6.14	5.55	4.58	22.00
7	127	F	*A/A*	5.87	8.48	7.83	5.61	11.33
14	129	F	*A/T*	6.28	8.47	11.35	6.58	8.67
2	130	F	*A/A*	6.93	6.59	4.00	4.56	13.67
2	131	M	*A/A*	6.34	7.55	8.58	6.37	9.33
10	132	M	*A/A*	6.86	6.08	10.28	6.51	6.67
2	133	M	*A/A*	6.79	6.34	9.02	5.77	11.33
3	136	F	*A/A*	5.73	5.90	6.43	4.75	11.00
3	137	F	*A/A*	5.51	6.48	4.74	4.49	19.67
12	139	F	*T/T*	5.43	6.82	12.19	10.26	8.33
3	140	M	*A/A*	8.05	8.84	6.28	5.72	12.67
5	142	M	*A/T*	6.09	8.43	9.43	7.11	13.33
5	144	F	*A/T*	4.20	5.26	7.67	4.69	20.67
11	145	F	*A/A*	5.04	5.61	10.41	7.20	5.67
11	146	M	*A/A*	8.84	7.83	9.39	6.60	1.33
5	147	M	*A/T*	5.40	6.60	7.27	7.37	12.00
11	149	M	*A/T*	8.46	9.24	13.31	12.69	2.00
14	151	M	*A/T*	5.86	6.61	9.49	6.99	3.67
9	152	F	*T/T*	5.32	6.49	8.95	7.87	7.33
15	154	F	*A/A*	6.71	7.29	8.52	6.87	19.33
16	156	F	*A/A*	8.61	6.72	7.95	6.27	10.33
15	157	M	*A/A*	8.42	9.42	10.69	7.23	9.67
14	158	F	*T/T*	6.60	8.19	9.79	8.07	9.00
9	159	M	*A/T*	7.49	6.89	10.00	8.49	3.00
13	160	M	*A/T*	9.29	10.67	13.69	11.08	4.00
3	161	M	*A/A*	7.38	6.91	6.96	6.12	5.67
9	162	F	*A/A*	6.93	6.20	8.12	5.99	6.67
14	163	M	*T/T*	7.41	10.16	11.42	8.11	3.17
13	164	M	*A/T*	8.13	8.82	15.58	18.53	2.17
3	165	F	*A/A*	6.86	8.37	6.59	4.95	13.33
9	166	F	*A/T*	6.45	9.32	7.46	6.44	8.00
8	167	F	*T/T*	5.59	7.21	9.18	4.85	10.33
15	168	M	*A/A*	9.38	6.66	7.13	6.54	8.67
16	169	F	*A/A*	6.04	6.41	7.85	6.74	10.33
16	170	F	*A/A*	6.04	7.87	8.46	6.70	20.00
15	171	M	*A/A*	6.65	6.89	9.12	9.51	6.67
16	172	M	*A/A*	8.05	7.81	9.40	8.07	7.67
15	173	F	*A/A*	6.18	7.70	10.71	7.43	15.67
4	174	F	*A/T*	5.36	8.03	7.93	7.76	15.33
3	175	F	*A/A*	5.96	6.88	6.57	4.48	12.67
3	176	M	*A/A*	8.18	7.09	7.61	5.68	14.00
16	177	M	*A/A*	6.92	9.32	12.28	10.35	7.00
8	178	F	*T/T*	6.07	6.34	9.99	6.47	7.33
3	179	M	*A/A*	8.84	7.84	7.15	4.31	8.00

Family	Tag	Sex	*PMCH*	Hip Roast	Shoulder Roast	Steak Shear Force SK	Steak Shear Force AB	Average Fat
3	180	M	*A/A*	7.63	6.98	7.69	5.41	9.33
8	181	F	*T/T*	3.72	6.48	7.62	•	8.00
16	182	M	*A/A*	7.57	7.41	7.75	8.24	5.67
9	185	M	*T/T*	6.90	9.39	10.17	7.72	5.33
16	186	F	*A/A*	6.08	7.97	6.19	6.29	11.00
3	187	F	*A/A*	5.73	7.75	5.79	5.30	15.67
8	189	F	*T/T*	5.77	8.39	6.85	3.21	8.33
8	190	F	*T/T*	6.15	7.35	9.89	6.80	8.33
16	191	M	*A/A*	7.82	8.99	10.33	9.76	7.00
8	192	M	*T/T*	9.24	7.72	9.39	9.73	3.33
4	193	M	*A/T*	9.86	9.58	7.02	5.16	6.67
11	195	F	*A/A*	6.74	6.75	7.09	5.98	4.00
8	197	M	*T/T*	7.78	7.18	9.08	6.99	3.33
10	198	M	*A/A*	10.14	6.66	6.65	6.20	6.67
4	199	M	*A/T*	7.26	8.29	5.97	4.65	7.33
10	200	M	*A/A*	7.94	8.96	7.07	6.63	9.00
11	201	M	*A/A*	6.74	9.45	11.03	9.32	5.00
13	202	F	*A/T*	6.64	9.05	11.57	7.81	8.00
13	203	F	*T/T*	7.00	9.35	10.90	6.88	7.17
11	204	F	*A/T*	8.19	8.39	8.61	5.74	6.33
11	205	M	*A/A*	9.96	10.37	10.71	6.21	1.50
17	206	M	*A/T*	7.55	8.84	11.35	7.65	14.00
17	207	F	*A/T*	7.27	6.41	7.45	4.46	18.33
13	208	M	*A/A*	6.67	11.33	12.42	6.45	2.17
17	209	M	*A/T*	7.34	10.14	13.29	11.44	14.67
15	210	M	*A/A*	9.43	7.84	6.99	7.40	9.33
17	212	M	*A/T*	6.86	9.02	11.40	11.77	8.67
17	213	M	*A/T*	7.98	8.57	13.30	5.10	11.67
15	214	F	*A/A*	7.08	6.95	6.10	4.38	16.00
2	341	M	*A/A*	9.02	5.93	7.05	8.38	5.67
1	342	M	*A/T*	5.79	5.87	7.64	5.77	5.00
1	392	F	*A/A*	5.68	5.51	8.17	5.18	13.67
2	398	F	*A/A*	6.45	5.62	7.21	6.44	18.33
1	402	F	*A/T*	7.15	7.55	7.50	6.60	14.00
1	403	F	*A/A*	7.54	6.81	7.31	7.07	9.67
1	404	F	*A/T*	8.18	6.96	7.41	6.13	7.00
1	405	F	*A/A*	7.13	7.75	7.38	7.32	11.00
1	406	F	*A/T*	5.64	7.90	8.85	7.39	10.00
2	408	F	*A/A*	6.02	6.26	6.02	7.00	15.67
1	414	F	*A/A*	6.42	6.38	9.45	8.40	9.67

CBRH trait values continued

Tag	Grade Fat	Palatability[1]	Initial Tenderness*	Overall Tenderness*
25	5	4.33	3.33	3.33
27	9	6.50	6.67	6.67
28	8	5.50	7.17	6.67
29	10	2.83	3.00	2.67
33	4	2.83	3.17	3.33
34	8	5.50	6.33	6.33
35	6	4.33	4.00	4.00
36	9	4.17	4.67	4.67
46	8	6.00	5.00	5.83
48	8	4.50	4.33	4.50
57	13	4.50	4.17	3.50
61	7	3.67	3.17	3.33
65	10	5.33	5.50	5.83
69	9	5.40	5.60	5.40
74	11	3.50	4.50	3.50
76	5	2.67	2.33	2.33
84	12	2.40	2.00	2.20
86	3	3.50	2.83	3.00
87	4	4.17	3.17	3.50
92	4	3.33	2.83	3.17
93	5	3.50	3.17	2.67
94	7	5.33	5.33	5.33
98	4	3.33	2.67	3.00
100	8	4.50	4.17	4.67
103	7	4.33	4.83	4.67
104	13	6.83	6.67	7.00
107	7	4.33	5.83	5.50
108	9	4.83	5.00	5.17
109	6	5.17	4.17	4.50
110	4	3.80	4.40	4.40
112	9	5.83	7.33	7.33
113	3	4.00	4.33	4.17
114	5	5.33	5.50	5.50
115	13	4.33	3.50	3.83
116	12	5.00	5.83	6.00
119	10	3.17	3.00	3.33
122	7	3.83	4.33	4.00
124	2	4.50	5.00	4.83
126	16	6.17	6.67	7.00
127	11	4.67	4.33	4.67
129	5	3.67	3.83	3.33
130	10	5.17	7.00	7.17
131	8	3.67	4.00	4.67
132	5	4.33	3.17	3.67

[1] Palatability, initial tenderness, and overall tenderness were evaluated by a consumer taste panel.

Tag	Grade Fat	Palatability	Initial Tenderness	Overall Tenderness
133	8	4.67	4.33	4.50
136	7	5.17	6.83	7.17
137	16	6.50	7.00	7.17
139	8	4.17	4.83	4.33
140	9	5.83	6.00	6.17
142	12	3.67	3.17	3.33
144	17	5.50	4.67	5.00
145	5	4.20	4.60	4.60
146	1	4.33	5.33	5.17
147	10	5.50	6.00	6.17
149	1	2.67	2.17	2.17
151	3	6.00	6.33	6.67
152	7	5.00	6.00	6.50
154	13	4.67	5.50	5.33
156	11	3.60	2.80	3.20
157	7	4.17	4.67	4.50
158	7	3.67	4.50	3.67
159	3	3.67	3.67	3.50
160	4	4.17	3.00	2.67
161	4	4.17	6.00	6.00
162	5	4.33	4.50	4.17
163	3	2.83	2.67	2.83
164	2	2.00	2.00	1.83
165	9	6.00	7.50	7.33
166	8	5.33	5.50	5.50
167	6	4.67	5.67	5.50
168	8	5.00	5.17	5.00
169	10	4.67	5.00	5.17
170	16	4.67	5.67	6.17
171	4	3.33	3.00	3.00
172	5	5.50	5.33	5.17
173	13	4.83	4.17	4.67
174	15	6.67	7.50	7.33
175	8	5.17	5.33	5.50
176	10	4.50	4.33	4.50
177	5	4.17	4.00	3.83
178	5	4.17	4.00	3.67
179	6	5.17	6.00	6.17
180	8	4.17	4.50	4.67
181	6	6.17	6.00	6.17
182	6	4.17	4.00	3.67
185	4	4.17	5.00	4.17
186	9	6.17	6.17	6.33
187	14	4.67	6.00	5.83
189	5	5.00	6.17	6.67
190	5	4.67	5.00	5.00
191	6	4.33	4.33	4.67
192	1	3.33	3.67	3.50

Tag	Grade Fat	Palatability	Initial Tenderness	Overall Tenderness
193	4	5.17	6.50	5.83
195	4	4.50	5.67	5.00
197	2	4.50	5.67	5.00
198	5	5.83	7.00	6.83
199	7	5.17	5.33	5.67
200	5	6.00	6.00	6.33
201	5	4.00	4.00	3.83
202	8	4.00	5.20	5.00
203	7	4.83	4.50	4.17
204	4	4.50	5.50	5.50
205	2	5.00	5.17	5.17
206	7	4.00	4.33	3.83
207	12	5.33	5.83	5.17
208	3	3.83	3.67	3.50
209	9	4.33	3.83	4.17
210	8	5.33	5.50	5.67
212	8	4.33	5.17	4.83
213	9	3.67	3.33	3.33
214	17	6.67	6.50	6.83
341	4	5.00	5.83	5.67
342	3	4.50	5.00	5.33
392	11	6.83	7.00	6.83
398	17	6.33	7.33	7.17
402	12	6.00	6.67	6.83
403	6	5.00	6.67	6.33
404	6	4.83	5.17	4.83
405	9	7.17	6.83	7.50
406	9	4.50	3.83	3.67
408	12	5.33	5.50	5.83
414	8	6.33	5.67	6.00

Appendix B Behaviour Population *PMCH* genotype and carcass fat measurements

lab	tag	*PMCH*	Average fat (mm)	Grade fat (mm)
06-001	12	*T/T*	10	8
06-002	7	*A/T*	4	4
06-003	5	*A/A*	5	5
06-004	20	*A/T*	8	7
06-005	25	*T/T*	7	5
06-006	19	*A/T*	9	7
06-007	23	*A/A*	12	10
06-008	18	*A/T*	13	11
06-010	3	*T/T*	8	5
06-011	21	*A/A*	14	10
06-012	33	*T/T*	14	13
06-013	26	*A/T*	7	5
06-014	4	*A/T*	9	7
06-015	15	*A/A*	6	5
06-016	17	*A/A*	10	8
06-017	14	*A/T*	3	2
06-018	29	*A/A*	14	13
06-019	13	*A/A*	10	9
06-020	2	*A/A*	12	11
06-022	8	*A/A*	7	6
06-023	1	*A/A*	9	8
06-024	10	*A/A*	12	10
06-025	30	*A/T*	16	14
06-026	28	*T/T*	8	7
06-027	32	*A/A*	11	9
06-028	9	*A/A*	16	15
06-029	31	*A/T*	8	6
06-030	24	*A/A*	14	12
06-031	22	*A/A*	6	4
06-032	11	*T/T*	7	5
06-033	6	*A/T*	5	5
06-034	65	*A/T*	6	5
06-035	41	*A/A*	14	12
06-036	39	*A/T*	3	2
06-037	64	*A/T*	8	7
06-040	50	*T/T*	16	11
06-041	55	*A/A*	9	8
06-042	52	*A/A*	8	9
06-043	54	*A/T*	5	4
06-044	51	*A/T*	18	20
06-045	48	*T/T*	7	7
06-046	40	*T/T*	5	4
06-047	45	*A/A*	6	6
06-048	43	*A/T*	7	7
06-049	49	*A/A*	10	9
06-050	61	*T/T*	8	7
06-051	59	*A/A*	5	4

lab	tag	*PMCH*	Average fat (mm)	Grade fat (mm)
06-052	38	*T/T*	8	6
06-053	46	*A/A*	6	5
06-054	53	*T/T*	15	14
06-055	67	*A/T*	9	5
06-056	56	*A/A*	10	7
06-057	47	*A/T*	11	9
06-058	57	*A/A*	19	20
06-059	63	*A/A*	3	3
06-060	58	*A/A*	14	14
06-061	34	*A/A*	9	7
06-062	42	*A/A*	14	12
06-063	62	*A/T*	12	10
06-064	35	*A/A*	10	8
06-065	36	*T/T*	5	5
06-066	60	*A/T*	12	8
06-067	44	*A/A*	7	5
06-068	66	*A/A*	11	8
06-069	37	*A/A*	4	4
06-070	76	*A/A*	15	14
06-071	81	*A/A*	13	12
06-072	78	*A/A*	10	8
06-073	97	*A/A*	7	5
06-074	79	*A/A*	7	5
06-075	86	*A/A*	12	10
06-076	96	*A/T*	5	3
06-077	68	*A/T*	7	6
06-078	87	*A/T*	13	10
06-079	93	*A/T*	5	4
06-080	69	*A/T*	16	12
06-082	71	*A/T*	12	9
06-083	99	*A/T*	7	6
06-084	94	*A/A*	11	9
06-085	90	*A/A*	10	8
06-086	95	*A/A*	5	4
06-087	73	*T/T*	15	16
06-088	70	*A/A*	6	5
06-089	77	*A/A*	4	2
06-090	85	*A/T*	5	4
06-091	83	*A/A*	16	13
06-092	82	*A/A*	7	4
06-093	84	*A/A*	16	12
06-094	91	*A/A*	7	5
06-095	88	*A/T*	13	12
06-097	89	*A/T*	11	9
06-098	100	*A/A*	4	3
06-100	80	*A/A*	11	7
06-101	75	A/T	5	4
06-102	110	A/A	7	4

lab	tag	*PMCH*	Average fat (mm)	Grade fat (mm)
06-103	109	*A/A*	10	10
06-104	116	*A/T*	11	10
06-105	118	*A/A*	11	9
06-106	133	*A/T*	5	4
06-107	126	*A/A*	9	8
06-108	112	*A/T*	12	11
06-109	120	*A/T*	10	8
06-110	124	*A/T*	14	10
06-111	104	*A/A*	11	10
06-112	113	*A/T*	7	5
06-113	122	*A/A*	11	11
06-114	121	*T/T*	8	6
06-115	117	*A/T*	10	8
06-116	115	*A/T*	8	7
06-117	129	*A/T*	7	5
06-118	130	*A/A*	8	7
06-119	127	*A/A*	9	7
06-120	132	*A/A*	14	13
06-121	108	*A/A*	9	7
06-122	106	*A/A*	5	5
06-123	105	*A/A*	14	12
06-124	101	*A/A*	11	8
06-125	119	*T/T*	9	8
06-126	111	*A/T*	9	8
06-127	123	*T/T*	8	6
06-128	125	*A/A*	11	9
06-129	128	*T/T*	13	10
06-130	107	*A/A*	7	5
06-131	102	*A/A*	14	12
06-132	131	*A/A*	5	4
06-133	114	*A/A*	11	9
06-134	103	*A/A*	13	12
06-136	147	*A/T*	8	7
06-137	164	*A/T*	7	8
06-138	146	*A/A*	12	13
06-139	162	*A/A*	9	7
06-140	144	*A/A*	17	15
06-141	163	*A/T*	9	8
06-142	148	*A/A*	9	7
06-143	158	*A/T*	11	10
06-144	140	*A/A*	10	8
06-145	145	*A/A*	9	7
06-146	136	*A/A*	14	15
06-147	155	*A/T*	8	6
06-148	142	*T/T*	9	7
06-149	152	*T/T*	9	7
06-150	159	*A/T*	7	5
06-151	138	*A/A*	12	10

lab	tag	*PMCH*	Average fat (mm)	Grade fat (mm)
06-152	139	*A/T*	14	12
06-153	156	*A/T*	12	10
06-154	135	*T/T*	7	5
06-155	137	*T/T*	6	5
06-156	166	*A/A*	12	9
06-157	165	*A/T*	9	7
06-158	149	*A/A*	4	3
06-159	151	*A/T*	15	15
06-160	143	*A/T*	9	6
06-161	161	*A/T*	6	5
06-162	160	*A/A*	10	9
06-163	141	*A/A*	11	7
06-164	153	*T/T*	8	7
06-165	134	*A/T*	4	3
06-166	154	*A/A*	6	5
06-167	157	*T/T*	5	4
06-168	150	*A/A*	13	12
06-169	221	*T/T*	8	6
06-170	218	*A/T*	19	17
06-171	202	*A/A*	6	5
06-172	201	*T/T*	2	1
06-173	223	*A/T*	10	7
06-174	203	*A/A*	6	5
06-175	216	*A/A*	5	4
06-176	211	*A/A*	6	4
06-177	210	*A/A*	13	12
06-178	215	*T/T*	5	4
06-179	200	*A/T*	10	7
06-180	209	*A/T*	8	7
06-181	212	*A/T*	11	9
06-182	206	*A/T*	9	8
06-183	225	*A/T*	16	17
06-184	229	*A/A*	10	11
06-185	204	*A/A*	8	7
06-186	222	*A/T*	8	6
06-187	219	*A/T*	15	16
06-188	205	*A/T*	5	4
06-189	213	*A/A*	10	7
06-190	208	*A/T*	8	6
06-191	224	*A/A*	6	5
06-192	226	*A/T*	10	9
06-193	217	*A/A*	15	12
06-194	227	*A/T*	7	5
06-195	207	*A/T*	9	6
06-196	220	*A/A*	10	8
06-197	214	*A/A*	5	5
06-198	228	*A/T*	8	7
06-199	180	*A/A*	16	15
06-200	184	*T/T*	10	8

lab	tag	*PMCH*	Average fat (mm)	Grade fat (mm)
06-201	188	*A/A*	16	14
06-202	186	*A/T*	6	5
06-203	194	*A/T*	12	10
06-205	174	*A/A*	3	3
06-206	170	*A/A*	15	14
06-207	185	*A/A*	12	11
06-208	196	*A/A*	16	15
06-209	182	*T/T*	13	14
06-210	171	*A/A*	9	8
06-211	183	*A/T*	7	5
06-212	173	*A/A*	10	9
06-213	181	*A/A*	10	8
06-214	191	*A/T*	7	5
06-215	187	*A/T*	10	8
06-216	199	*A/T*	6	5
06-217	197	*A/T*	7	6
06-218	193	*A/A*	7	5
06-219	189	*T/T*	14	12
06-220	178	*A/T*	6	5
06-221	168	*A/A*	12	11
06-223	195	*A/A*	11	11
06-224	167	*A/A*	8	7
06-225	175	*A/A*	3	2
06-226	169	*A/T*	12	9
06-227	177	*A/T*	4	4
06-228	192	*A/T*	6	5
06-230	198	*A/T*	7	5
06-231	190	*A/T*	14	15
06-232	172	*A/T*	9	7
06-233	237	*A/T*	20	18
06-234	258	*A/A*	16	15
06-235	265	*A/A*	9	8
06-236	249	*A/T*	9	7
06-237	236	*A/T*	9	8
06-238	256	*A/A*	10	9
06-240	239	*A/T*	14	12
06-241	230	*A/T*	11	9
06-242	232	*A/A*	19	19
06-243	233	*T/T*	7	6
06-244	251	*A/T*	15	13
06-245	259	*A/T*	13	13
06-246	243	*A/T*	12	12
06-247	231	*A/A*	13	12
06-248	252	*A/T*	14	13
06-250	253	*A/T*	6	5
06-251	246	*A/T*	14	13
06-252	241	*A/A*	13	13
06-253	254	*A/T*	12	10
06-254	257	*A/T*	11	9

lab	tag	*PMCH*	Average fat (mm)	Grade fat (mm)
06-255	267	*A/T*	9	8
06-256	242	*A/A*	16	14
06-257	247	*A/A*	11	9
06-258	255	*A/A*	8	5
06-259	260	*A/A*	10	9
06-260	234	*T/T*	9	7
06-261	264	*A/T*	9	8
06-262	266	*A/T*	12	10
06-263	235	*A/A*	14	11
06-264	268	*A/A*	16	14
06-265	250	*A/T*	13	8
06-266	334	*T/T*	4	4
06-267	330	*T/T*	10	8
06-269	333	*A/T*	8	7
06-270	328	*A/A*	9	8
06-271	322	*A/T*	4	3
06-272	307	*A/A*	8	5
06-273	336	*A/T*	10	10
06-274	337	*T/T*	9	7
06-276	315	*A/A*	7	6
06-277	326	*A/A*	11	8
06-278	309	*A/A*	9	7
06-279	325	*A/A*	9	7
06-280	327	*A/T*	5	4
06-282	320	*A/T*	13	11
06-283	319	*A/A*	14	12
06-284	323	*A/A*	7	8
06-285	335	*A/A*	16	16
06-286	312	*A/A*	10	8
06-287	340	*A/A*	10	9
06-288	324	*A/A*	19	18
06-289	329	*A/A*	9	7
06-290	308	*A/T*	10	8
06-291	311	*A/T*	10	7
06-292	313	*A/A*	8	6
06-293	310	*T/T*	9	7
06-294	332	*A/A*	11	11
06-295	331	*A/T*	9	8
06-296	316	*A/T*	6	6
06-297	318	*A/T*	14	11
06-298	338	*A/A*	7	4
06-299	321	*A/A*	12	12
06-300	296	*A/T*	13	11
06-301	301	*A/A*	17	15
06-302	294	*A/A*	10	7
06-303	292	*A/A*	14	14
06-304	289	*A/A*	9	8
06-305	263	*A/T*	13	13
06-306	290	*A/A*	12	11

lab	tag	*PMCH*	Average fat (mm)	Grade fat (mm)
06-307	277	*T/T*	14	10
06-308	304	*T/T*	8	8
06-309	281	*A/A*	12	10
06-310	293	*A/T*	13	12
06-311	269	*A/T*	15	13
06-312	240	*A/A*	5	4
06-313	288	*A/T*	12	12
06-314	274	*A/A*	6	4
06-315	272	*A/A*	15	14
06-316	271	*A/A*	16	16
06-318	248	*A/T*	8	7
06-319	287	*A/T*	6	5
06-320	280	*A/A*	11	8
06-321	285	*A/T*	16	16
06-322	283	*A/T*	13	10
06-323	295	*T/T*	14	12
06-324	245	*A/A*	13	11
06-325	278	*A/T*	7	5
06-326	291	*A/A*	6	5
06-327	300	*T/T*	9	7
06-328	270	*A/T*	13	12
06-329	305	*A/A*	11	8
06-330	303	*T/T*	6	5
06-331	273	*A/T*	13	9
06-332	261	*A/A*	14	13
06-333	275	*A/T*	3	3
06-334	348	*A/T*	4	4
06-335	366	*A/A*	8	7
06-336	365	*A/T*	10	8
06-337	350	*A/A*	10	8
06-338	345	*A/A*	12	9
06-339	359	*A/A*	12	10
06-340	347	*A/T*	16	14
06-341	361	*A/T*	9	9
06-342	346	*T/T*	2	2
06-343	372	*A/T*	7	7
06-344	356	*A/A*	4	4
06-345	362	*A/T*	4	2
06-346	369	*A/A*	12	10
06-347	352	*T/T*	10	8
06-348	374	*A/T*	12	10
06-349	373	*A/T*	7	5
06-350	355	*A/A*	10	8
06-351	349	*A/A*	9	8
06-352	353	*A/A*	13	11
06-353	343	*A/A*	6	6
06-354	360	*A/A*	12	9
06-355	354	*A/A*	12	9
06-356	351	*A/A*	5	4

lab	tag	*PMCH*	Average fat (mm)	Grade fat (mm)
06-358	370	*A/T*	11	10
06-359	341	*A/T*	6	5
06-360	358	*A/T*	9	7
06-361	371	*A/A*	5	4
06-362	344	*A/A*	9	7
06-363	342	*A/T*	9	7
06-364	363	*A/A*	17	16
06-365	364	*A/T*	9	7
06-367	298	*A/T*	10	7
06-368	279	*A/A*	5	5
06-369	385	*A/T*	11	9
06-370	276	*A/T*	8	6
06-371	384	*A/T*	9	7
06-372	388	*A/T*	16	16
06-373	394	*A/A*	9	7
06-376	397	*T/T*	4	4
06-377	282	*A/T*	24	23
06-378	378	*A/T*	15	12
06-379	375	*A/A*	5	4
06-380	400	*A/T*	11	10
06-381	379	*A/T*	12	12
06-382	386	*A/T*	12	11
06-383	286	*A/A*	10	8
06-384	391	*A/A*	5	4
06-385	377	*A/T*	8	7
06-386	398	*A/A*	12	10
06-387	390	*A/T*	7	5
06-388	299	*A/A*	6	5
06-389	399	*A/A*	9	7
06-390	382	*T/T*	7	5
06-391	376	*A/T*	8	7
06-392	383	*T/T*	12	10
06-393	297	*A/T*	13	13
06-394	393	*A/A*	6	5
06-395	381	*A/T*	10	7
06-396	389	*A/A*	7	6
06-397	396	*A/T*	9	8
06-398	387	*A/T*	9	9
06-399	302	*T/T*	4	4
06-400	395	*A/T*	3	2
06-401	392	*A/T*	9	8

Appendix C Purebred Bull *PMCH* genotype and breed[2]

Lab	Tag	*PMCH*	Breed	Lab	Tag	*PMCH*	Breed
93-001	303	*A/A*	Angus	93-053	323	*A/T*	Angus
93-003	287	*A/A*	Angus	93-058	313	*A/A*	Angus
93-004	235	*A/T*	Charolais	93-061	291	*A/A*	Angus
93-005	317	*A/A*	Angus	93-062	249	*A/T*	Charolais
93-006	325	*A/A*	Angus	93-063	268	*A/T*	Charolais
93-007	248	*A/A*	Charolais	93-065	257	*A/T*	Charolais
93-008	273	*A/A*	Charolais	93-067	230	*A/A*	Charolais
93-010	314	*A/A*	Angus	93-068	261	*T/T*	Charolais
93-011	305	*A/T*	Angus	93-069	272	*A/T*	Charolais
93-012	233	*A/A*	Charolais	93-070	229	*A/A*	Charolais
93-013	256	*A/T*	Charolais	93-071	238	*A/A*	Charolais
93-015	252	*A/A*	Charolais	93-072	274	*A/T*	Charolais
93-016	277	*A/A*	Charolais	93-073	232	*A/A*	Charolais
93-017	253	*A/A*	Charolais	93-074	245	*A/T*	Charolais
93-019	251	*A/A*	Charolais	93-075	286	*A/A*	Angus
93-020	259	*A/T*	Charolais	93-076	297	*A/A*	Angus
93-021	288	*A/A*	Angus	93-077	265	*A/A*	Charolais
93-022	300	*A/A*	Angus	93-079	263	*A/A*	Charolais
93-023	320	*A/A*	Angus	93-080	250	*A/T*	Charolais
93-024	296	*A/A*	Angus	93-081	242	*A/T*	Charolais
93-025	301	*A/A*	Angus	94-001	366	*A/T*	Charolais
93-026	298	*A/A*	Angus	94-002	368	*A/A*	Charolais
93-027	311	*A/A*	Angus	94-003	349	*A/A*	Simmental
93-028	322	*A/T*	Angus	94-004	387	*A/T*	Charolais
93-029	307	*A/T*	Angus	94-005	330	*A/A*	Charolais
93-030	293	*A/A*	Angus	94-006	427	*A/T*	Simmental
93-031	312	*A/A*	Angus	94-007	331	*A/T*	Charolais
93-032	309	*A/T*	Angus	94-008	391	*A/T*	Charolais
93-033	289	*A/A*	Angus	94-009	365	*A/A*	Charolais
93-034	319	*A/A*	Angus	94-010	350	*A/A*	Simmental
93-035	264	*A/A*	Charolais	94-011	334	*A/T*	Charolais
93-036	304	*A/T*	Angus	94-012	332	*A/T*	Charolais
93-038	318	*A/A*	Angus	94-013	367	*A/A*	Charolais
93-039	243	*A/T*	Charolais	94-014	363	*A/A*	Simmental
93-040	260	*A/T*	Charolais	94-015	347	*A/T*	Simmental
93-042	237	*A/A*	Charolais	94-016	358	*A/T*	Angus
93-043	340	*A/A*	Angus	94-017	378	*A/A*	Hereford
93-044	247	*T/T*	Charolais	94-020	343	*A/A*	Angus
93-045	299	*A/A*	Angus	94-021	408	*A/A*	Hereford
93-046	231	*A/A*	Charolais	94-022	356	*A/T*	Hereford
93-047	295	*A/A*	Angus	94-023	381	*A/T*	Hereford
93-048	310	*A/A*	Angus	94-035	406	*A/A*	Hereford
93-051	308	*A/A*	Angus	94-036	400	*A/A*	Angus
93-052	302	*A/A*	Angus	94-037	359	*A/A*	Angus

[2] These bulls were initially used to study the accuracy of Real-Time ultrasound to predict lean meat yield in yearling bulls (Bergen *et al.* 1996).

Lab	Tag	*PMCH*	Breed	Lab	Tag	*PMCH*	Breed
94-038	351	*A/T*	Hereford	94-080	389	*A/A*	Charolais
94-039	399	*A/A*	Angus	94-081	390	*A/T*	Charolais
94-040	360	*A/T*	Hereford	94-082	396	*A/A*	Charolais
94-041	337	*A/T*	Angus	94-083	393	*A/T*	Charolais
94-042	369	*A/T*	Angus	94-084	425	*T/T*	Simmental
94-043	338	*A/A*	Angus	94-085	431	*A/T*	Simmental
94-044	336	*A/A*	Angus	94-087	342	*A/A*	Angus
94-045	374	*A/A*	Hereford	94-088	341	*A/A*	Angus
94-052	385	*T/T*	Angus	94-089	398	*A/T*	Angus
94-053	407	*A/A*	Hereford	94-090	344	*A/T*	Angus
94-054	384	*A/A*	Angus	94-091	372	*T/T*	Angus
94-055	401	*A/A*	Hereford	94-092	402	*A/A*	Hereford
94-057	404	*A/T*	Hereford	94-093	380	*T/T*	Hereford
94-058	355	*A/T*	Hereford	94-094	379	*T/T*	Hereford
94-059	370	*T/T*	Angus	94-095	382	*A/A*	Angus
94-060	429	*T/T*	Simmental	94-096	354	*A/T*	Hereford
94-066	326	*A/T*	Charolais	94-097	339	*A/A*	Angus
94-067	362	*A/T*	Simmental	94-098	432	*A/T*	Simmental
94-069	346	*A/T*	Simmental	94-099	376	*A/A*	Hereford
94-070	377	*A/A*	Hereford	94-100	394	*A/A*	Charolais
94-071	403	*A/T*	Hereford	94-101	392	*A/T*	Charolais
94-072	329	*A/A*	Charolais	94-102	430	*A/T*	Simmental
94-073	383	*A/A*	Angus	94-103	361	*T/T*	Simmental
94-074	333	*A/A*	Charolais	94-104	364	*T/T*	Simmental
94-075	424	*T/T*	Simmental	94-105	348	*A/T*	Simmental
94-076	388	*A/A*	Charolais	94-107	335	*A/A*	Angus
94-077	328	*A/T*	Charolais	94-108	352	*T/T*	Hereford
94-078	345	*T/T*	Simmental	94-110	373	*T/T*	Angus
94-079	428	*A/T*	Simmental	94-111	357	*A/T*	Hereford

Appendix D DNA extraction methods

CBRH and Bos indicus DNA extraction (Schmutz *et al*. 1995):

From Semen: One straw of semen was thawed to room temperature and emptied into a 10 ml tube. It was then washed with 1X SSC and 10 mM EDTA, followed by centrifugation at 3000 rpm for 3 min, three to four times. The supernatant was removed from the sample, and the pellet was resuspended in 500 μl of 1X TE. Six μl of proteinase K (20 mg/ml) and 10 μl of 20% SDS were added and incubated for 1 h at 60 °C. After the incubation period the tube was filled with 1X SSC with 10 mM EDTA and centrifuged at 12,000 rpm for 3 min. The supernatent was removed from the pellet, and 40 μl of proteinase K and 450 μl of semen extraction buffer were added (100 mM Tris-HCl pH 8.3, 10 mM EDTA, 500 mM NaCl, 1% SDS, and 2% mercaptoethanol). This solution was then incubated at 60 °C overnight. The following morning, 20 μl of proteinase K (20 mg/ml) was added, and the solution was incubated overnight again. Phenol/chloroform extraction and ethanol precipitation were then performed. The DNA pellet was stored at 4 °C until needed for PCR.

CBRH and Behaviour Population DNA extraction (Montgomery & Sise 1990):

White blood cells were collected from whole blood following lysis of the red blood cells in 2.5 volumes of cold sterile lysing solution (150 mM NH_4Cl, 10 mM KCl, and 0.1 mM EDTA). The solutions were held on ice, and red blood lysis observed by a change in the color of the solution from blood red to a dark clear red, 2-10 min after the addition of the lysing solution. White cells were then harvested by spinning at a relative centrifugal force (RCF) of 2000 for 10 min at 4 °C. Pelleted white cells were

resuspended with a pasteur pipette and washed twice in 10 ml of Tris buffered saline (140 mM NaCl, 0.5 mM KCl, 0.25 mM Tris HCl pH 7.4). Cells were pelleted between washes in a bench-top centrifuge spun at an approximate RCF of 1000 for 3 min.

Washed white blood cells were completely resuspended by vigorous vortexing in 9 ml of TE (0.1 mM EDTA, 10 mM Tris-HCl pH 8.0) so that no cell clumps remained. Fifty µl of proteinase K (10 mg/ml) and 0.5 ml 0.5 M EDTA, pH 8.0 were added and the tubes mixed. Five hundred μl of 10% SDS was added while gently swirling the tubes. The tubes were then incubated at 50 °C in a water bath with occasional mixing for 3 h.

Following proteinase K digestion, 4.3 ml of a saturated NaCl solution was added, the tubes were shaken vigorously for 30 s, and spun at RCF 2000 for 10 min. The supernatant containing DNA was transferred to a clean glass tube and two volumes of 95% ethanol were added. The DNA was spooled out using a sealed pasteur pipette and washed in 70% ethanol. The DNA was dried and resuspended in 200-400 µl of TE. The tubes containing DNA in TE were placed on a mixing wheel in a cold room for at least 24 h to ensure resuspension was complete. The concentration of DNA was measured in a scanning spectrophotometer.

Purebred Bull DNA extraction (Fitzsimmons *et al.* 1998):

DNA was obtained form whole blood via a phenol/chloroform extraction performed on an Automated Applied Biosystems 340A Nucleic Acid Extractor.

Appendix E Total RNA extraction and cDNA synthesis methods

Tissue sample collection: Fifteen tissues were collected from a 5-week-old Holstein bull calf and a 19-month-old crossbred *Bos taurus* steer immediately after death and snap frozen in liquid nitrogen. Each tissue was pulverized in an RNA-free area using a mortar and pestle for RNA extraction.

Assorted tissues were collected from the Holstein-cross calves shortly following death and placed in RNA*later*® (Ambion) for subsequent RNA extraction.

Complementary DNA synthesis (Goodall and Schmutz 2007): Total RNA was isolated from the biopsies and other tissue samples using total RNA isolation reagent (TRIzol: Gibco). A DNA digest was then performed and included approximately 4ug of total RNA, 1 μl of 10X Reaction Buffer, and 1 μl of *DNaseI* (Gibco). The mixture was incubated at room temperature for 15 min. One μl of 25 mM EDTA was added and the mixture was incubated at 65 °C for 15 min then placed on ice for 1 min, following which the mixture was briefly centrifuged. Synthesis of cDNA was then performed. 1 μl of oligo(dT) primer (Gibco) was added and the mixture was incubated at 65 °C for 10 min and placed on ice for 1 min. Two μl of 10X RT buffer, 4 μl of 25 mM $MgCl_2$, 1 μl of 10 mM dNTPs, 2 μl of 0.1 mM DTT, and 1 μl of RNAseOUT™ (Invitrogen) were then added to the mixture and incubated at 42 °C for 2 min. One μl of SuperscriptII RT™ (Invitrogen) was added and incubated at 42 °C for 60 min. The reaction was the incubated at 70 °C for 15 min and chilled on ice. The mixture was then centrifuged briefly and 1 μl of *RNase H* (Invitrogen) was added, followed by incubation at 37 °C for

20 min. The mixture was then divided into 5 μl aliquots and stored either at 4 °C (for immediate use) or at -80 °C (for long-term storage). Each 5 μl aliquot was diluted with 30 μl of 1X TE (pH 8.0) before use as a template for PCR.

Appendix F CRIMAP results of microsatellite and *PMCH* genotype linkage mapping in the Canadian Beef Reference Herd

Sex-averaged mapping results from CRIMAP on BTA5

Microsatellite/gene	Recombination fraction	Distance between markers (cM)	Final placement (cM)
BM6026			0.0
	0.21	22.3	
NTS			22.3
	0.05	4.9	
RM103			27.2
	0.19	20.2	
BL37			47.3
	0.10	10.0	
MAF23			57.3
	0.13	13.7	
PMCH			71.1
	0.07	6.8	
BM1819			77.9
	0.42	60.5	
BMS1658			138.4
	0.09	9.0	
BMS597			147.5
	0.13	13.3	
ETH2			160.8

Appendix G Output generated by the Neural Network Promoter Prediction program

Start End Score Promoter Sequence
63 113 0.91
AAGCTGGAAAATATATAAAGGCAAGAATCATTTACAAAGCAGGATGACTG
277 327 0.94
GACATGGTATTTAAAACGTTGAGGCTGGGGAAAGCCTTTCAGAAGGAAGA
359 409 0.86
TGGAGCAATATAAAAATGATGAGAGCAGTTTCATGAATGATGAAGAAAAC
649 699 0.89
TTTCTTAAAGGGTAAAAACAGTGCAGGGCATATTTAAATTGATCAATAAG

Appendix H Input to P-match and output generated

Input sequences:

Charolais 12:

```
TTGGTTTCTA TCTGATGAGT CATTTCTAAA ATGATGAAAG TTTTTCAAGT
GCTTTCTATT CAAGCTGGAA AATATATAAA GGCAAGAATC ATTTACAAAG
CAGGATGACT GAGAAATTTC ACTTCATTTT ATACATCCTT GTTTGACTCT
ATGCAAACAT CAAACTAAGG ATG
```

Simmental 24:

```
TTGGTTTCTA TCTGATGAGT CATTTCTAAA ATGATGTAAG TTTTTCAAGT
GCTTTCTATT CAAGCTGGAA AATATATAAA GGCAAGAATC ATTTACAAAG
CAGGATGACT GAGAAATTTC ACTTCATTTT ATACATCCTT GTTTGACTCT
ATGCAAACAT CAAACTAAGG ATG
```

P-match output

Charolais 12:

These are your search results from Fri, 5.5.2006 – 23:43 MEZ for the following search: CCA12-matrix

Search for sites by WeightMatrix library: matrixTFP60pm.dat

Sequence file: CCA12.seq

Matrix groups: vertebrates

Cut-offs: to minimize false negative matches

Scanning sequence ID: CCA12_0

FFF sequence: 1, searchname: CCA12-matrix, login name: scf651

Position (strand)	Core score	Matrix score	Sequence (always the (+)-strand is shown)	Factor name
2 (+)	1.000	0.871	ggttgGTTTC	c-Rel
2 (-)	0.979	0.966	GGTTGgtttc	c-Rel
6 (-)	1.000	0.979	GGTTTctatc	c-Rel
10 (-)	1.000	0.931	tcTATCTgatg	Evi-1
15 (+)	1.000	0.960	ctgaTGAGTcat	CREB
16 (+)	1.000	1.000	tGATGAgtcat	NF-E2
17 (+)	0.975	0.910	gatgAGTCAtt	Evi-1
18 (-)	0.917	0.090	atgagTCATTt	NF-E2
30 (+)	0.909	0.858	taaaaTGATG**a**agtt	ARP-1
34 (+)	0.976	0.911	atgaTG**A**AAgtt	CREB
39 (+)	0.988	0.968	g**a**aaGTTTTtca	HFH-1
43 (-)	0.786	0.850	GTTTTtcaag	c-Rel
47 (+)	0.963	0.892	ttcaAGTGCtt	Evi-1
62 (+)	0.975	0.892	ttcaAGCTGga	Evi-1
74 (-)	0.984	0.975	aaTATATaaag	Evi-1
85 (-)	0.971	0.840	GCAAGaatca	c-Rel
87 (-)	0.988	0.897	aagAATCAttta	CREB
95 (+)	0.786	0.851	tttacAAAGC	c-Rel
100 (+)	0.933	0.821	aaagcaGGATGactga	Elk-1
106 (-)	0.793	0.840	GGATGactga	c-Rel
107 (+)	0.968	0.899	gatgACTGAga	Evi-1
107 (-)	0.975	0.911	gaTGACTgaga	Evi-1
107 (-)	0.772	0.841	GATGActgag	c-Rel
117 (-)	0.976	0.908	aaaTTTCActtc	CREB
125 (-)	0.999	0.899	cttcattttatacatCCTTGtttgactc	BSAP
130 (+)	0.972	0.961	ttttATACAtc	Evi-1
131 (+)	0.798	0.864	tttatACATC	c-Rel
132 (+)	0.793	0.855	ttataCATCC	c-Rel
133 (-)	0.814	0.853	TATACatcct	c-Rel
144 (-)	1.000	0.814	gtttgcatctatGCAAAcatcaaactaag	Myogenin/NF-1
147 (+)	0.977	0.871	tgactCTATGcaaaca	ARP-1
149 (-)	0.972	0.903	acTCTATgcaa	Evi-1

FFF sequence: 2, searchname: CCA12-matrix, login name: scf651

Total sequences length=173

Total number of sites found=32

Frequency of sites per nucleotide=0.185

Simmental 24:
These are your search results from Fri, 5.5.2006 – 23:39 MEZ for the following search: CCA24-matrix
Search for sites by WeightMatrix library: matrixTFP60pm.dat
Sequence file: CCA12.seq
Matrix groups: vertebrates
Cut-offs: to minimize false negative matches
Scanning sequence ID: CCA24_0
FFF sequence: 1, searchname: CCA24-matrix, login name: scf651

Position (strand)	Core score	Matrix score	Sequence (always the (+)-strand is shown)	Factor name
2 (+)	1.000	0.871	ggttgGTTTC	c-Rel
2 (-)	0.979	0.966	GGTTGgtttc	c-Rel
6 (-)	1.000	0.979	GGTTTctatc	c-Rel
10 (-)	1.000	0.931	tcTATCTgatg	Evi-1
15 (+)	1.000	0.960	ctgaTGAGTcat	CREB
16 (+)	1.000	1.000	tGATGAgtcat	NF-E2
17 (+)	0.975	0.910	gatgAGTCAtt	Evi-1
18 (-)	0.917	0.909	atgagTCATTt	NF-E2
33 (+)	1.000	0.993	aatgATG**T**Aagta	E4BP4
34 (-)	0.939	0.962	atgatG**T**AAG	HLF
39 (+)	0.988	0.934	g**t**aaGTTTTtca	HFH-1
43 (-)	0.786	0.850	GTTTTtcaag	c-Rel
47 (+)	0.963	0.892	ttcaAGTGCtt	Evi-1
62 (+)	0.975	0.892	ttcaAGCTGga	Evi-1
74 (-)	0.984	0.975	aaTATATaaag	Evi-1
85 (-)	0.971	0.840	GCAAGaatca	c-Rel
87 (-)	0.988	0.897	aagAATCAttta	CREB
95 (+)	0.786	0.857	tttacAAAGC	c-Rel
100 (+)	0.933	0.821	aaagcaGGATGactga	Elk-1
106 (-)	0.793	0.840	GGATGactga	c-Rel
107 (+)	0.968	0.899	gatgACTGAga	Evi-1
107 (-)	0.772	0.841	GATGActgag	c-Rel
107 (-)	0.975	0.911	gaTGACTgaga	Evi-1
117 (-)	0.976	0.908	aaaTTTCActtc	CREB
125 (-)	0.999	0.899	cttcattttatacatCCTTGtttgactc	BSAP
130 (+)	0.972	0.961	ttttATACAtc	Evi-1
131 (+)	0.798	0.864	tttatACATC	c-Rel
132 (+)	0.793	0.855	ttataCATCC	c-Rel
133 (-)	0.814	0.853	TATACatcct	c-Rel
144 (-)	1.000	0.814	gtttgactctatGCAAAcatcaaactaag	Myogenin/NF-1
147 (+)	0.977	0.871	tgactCTATGcaaaca	ARP-1
149 (-)	0.972	0.903	acTCTATgcaa	Evi-1

FFF sequence: 2, searchname: CCA24-matrix, login name: scf651
Total sequences length=173
Total number of sites found=32
Frequency of sites per nucleotide=0.185